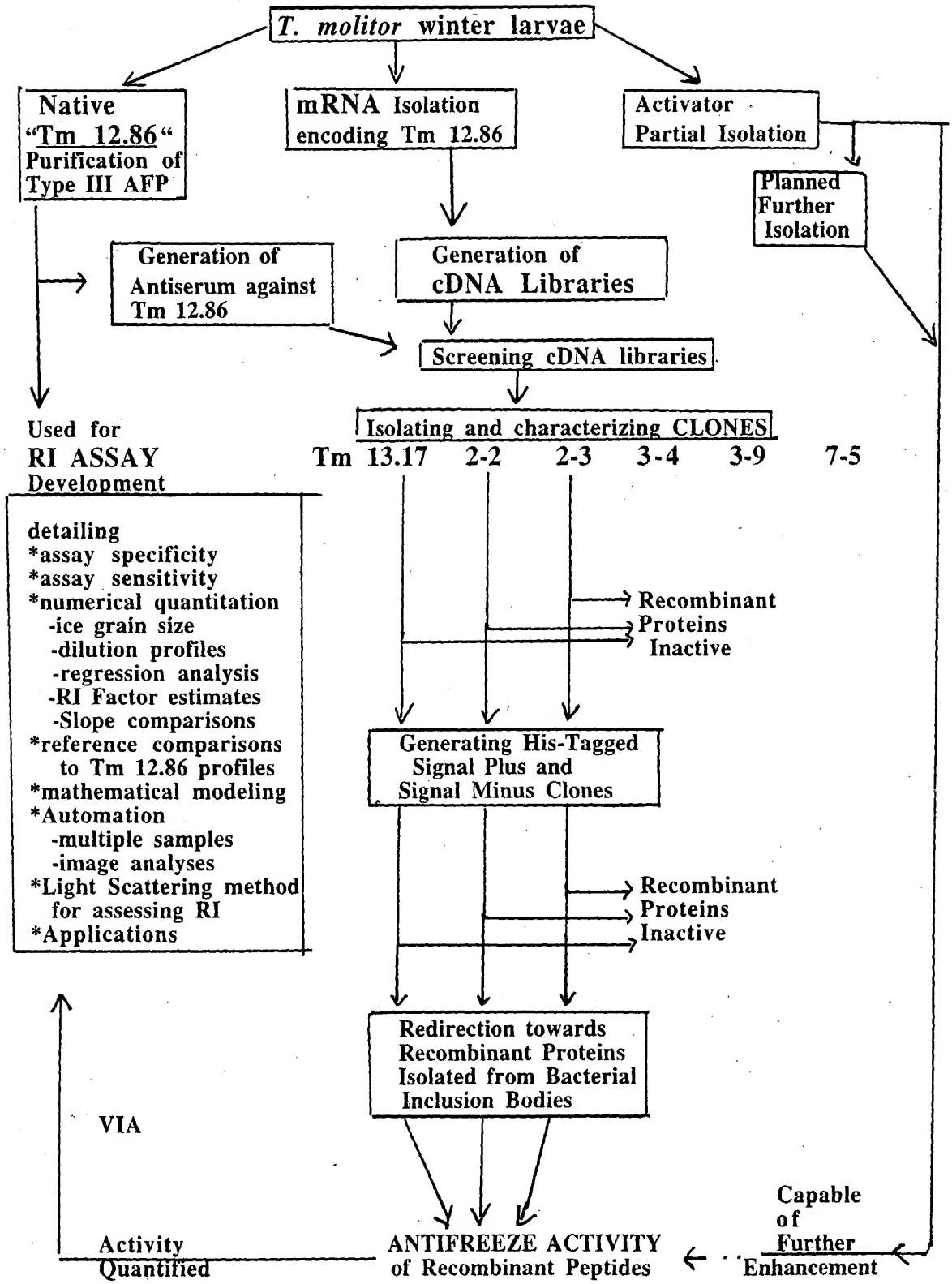
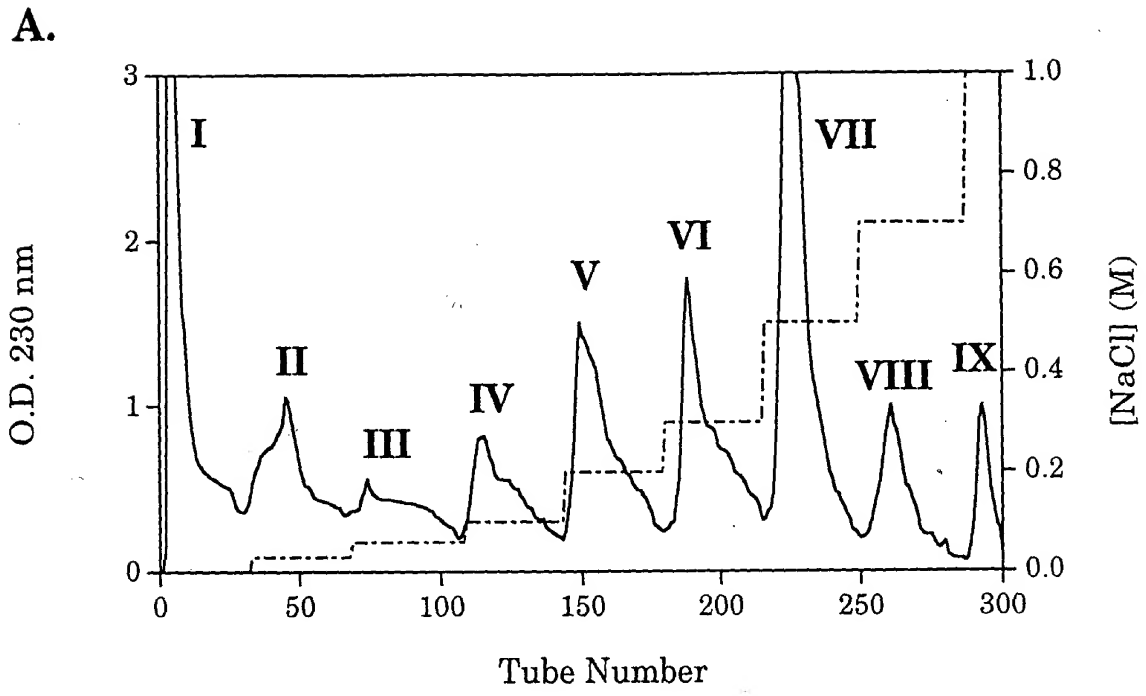

T. molitor winter larvae
Native "Tm 12.86" Purification of Type III AFP
mRNA Isolation encoding Tm 12.86
Activator Partial Isolation
Planned Further Isolation
Generation of Antiserum against Tm 12.86
Generation of cDNA Libraries
Screening cDNA libraries
Isolating and characterizing CLONES
Tm 13.17 2-2 2-3 3-4 3-9 7-5
Used for RI ASSAY Development
detailing
*assay specificity
*assay sensitivity
*numerical quantitation
-ice grain size
-dilution profiles
-regression analysis
-RI Factor estimates
-Slope comparisons
*reference comparisons to Tm 12.86 profiles
*mathematical modeling
*Automation
-multiple samples
-image analyses
*Light Scattering method for assessing RI
*Applications
Recombinant Proteins Inactive
Generating His-Tagged Signal Plus and Signal Minus Clones
Recombinant Proteins Inactive
Redirection towards Recombinant Proteins Isolated from Bacterial Inclusion Bodies
VIA
Activity Quantified
ANTIFREEZE ACTIVITY of Recombinant Peptides
Capable of Further Enhancement

FIG 1.0


A.
O.D. 230 nm
[NaCl] (M)
Tube Number
I
II
III
IV
V
VI
VII
VIII
IX
0
1
2
3
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300

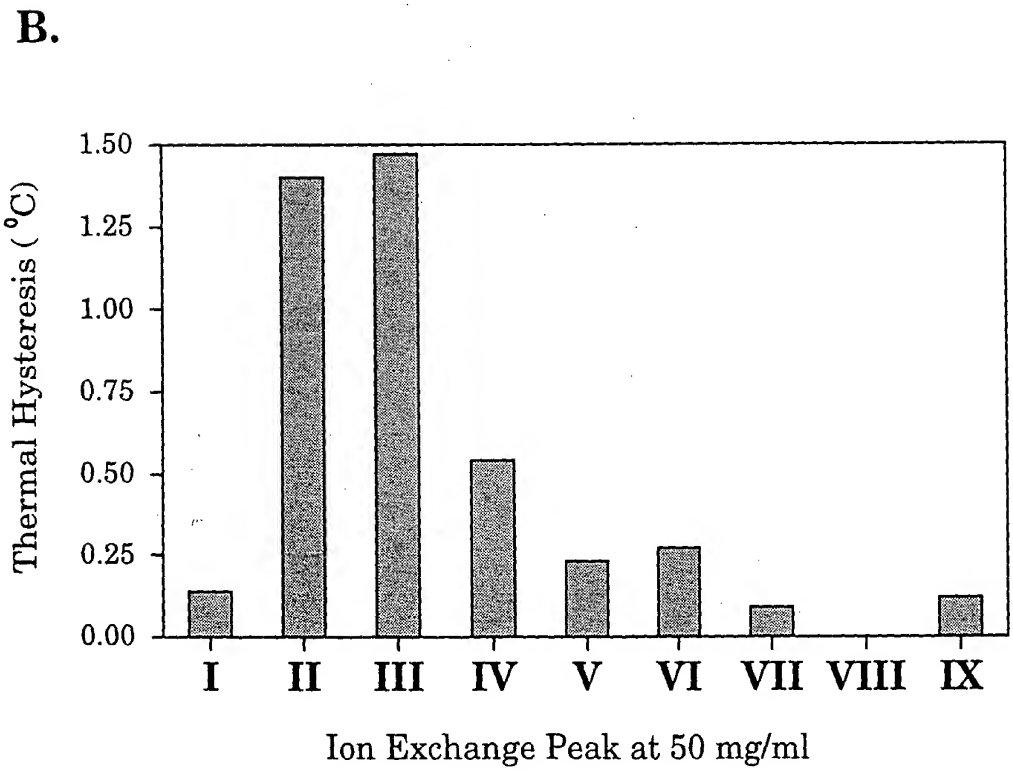

B.
Thermal Hysteresis (°C)
Ion Exchange Peak at 50 mg/ml
0.00
0.25
0.50
0.75
1.00
1.25
1.50
I
II
III
IV
V
VI
VII
VIII
IX

FIG 1.1

T02090 8hE92860

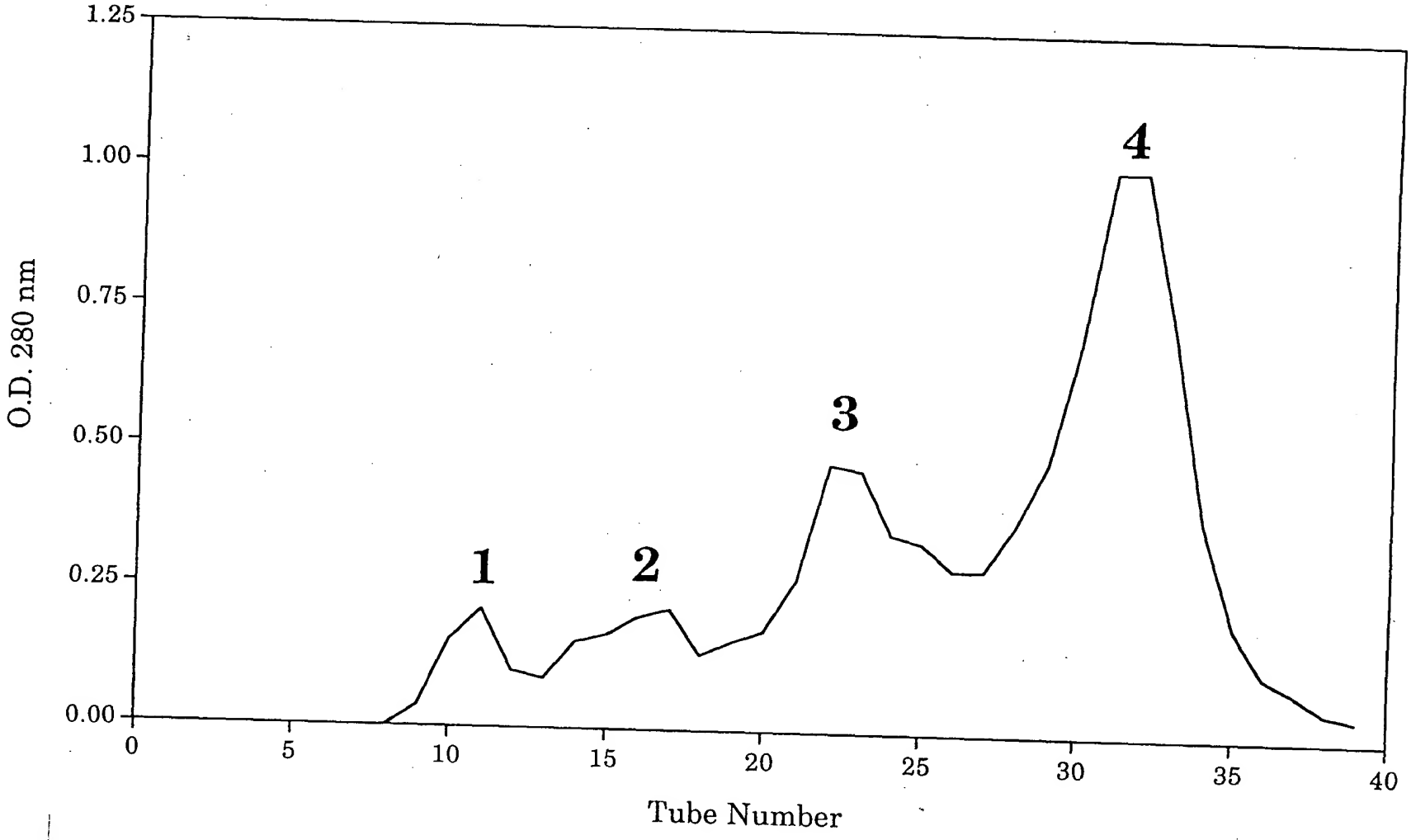



**FIG 1.2**

EP 0 360 848 A2

FIG 1.3

12.5 25

FIG 1.4

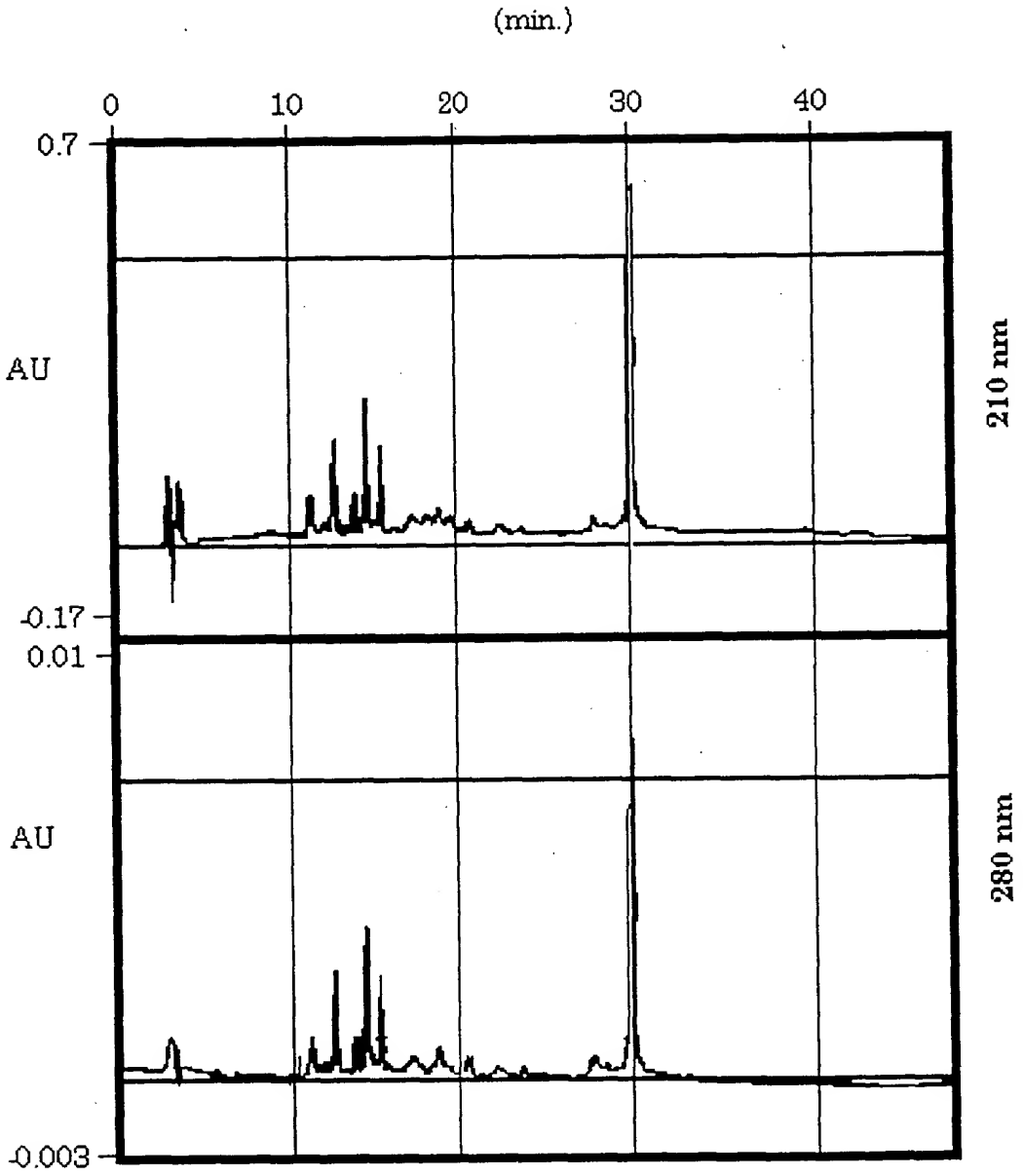



FIG 1.5

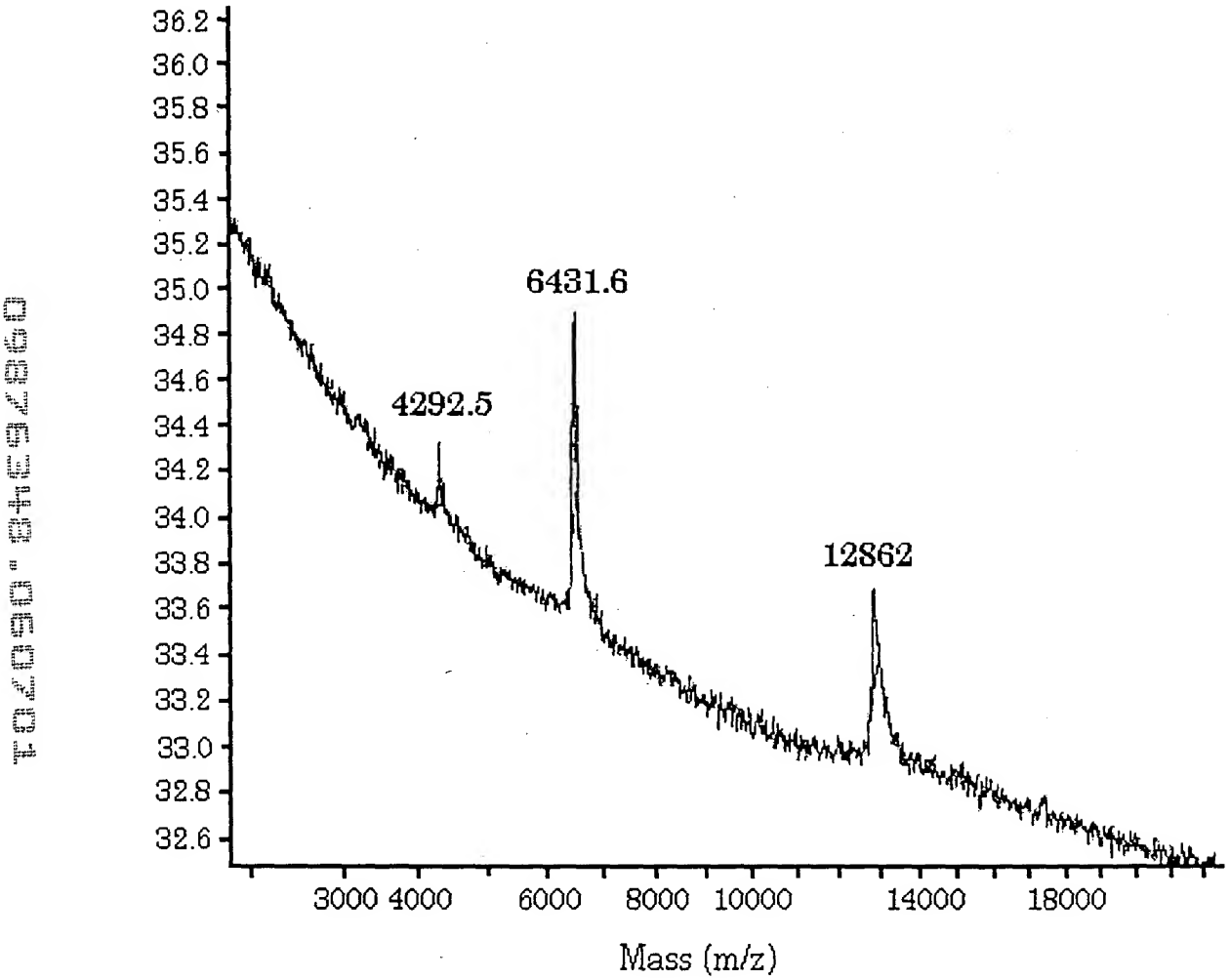

36.2
36.0
35.8
35.6
35.4
35.2
35.0
34.8
34.6
34.4
34.2
34.0
33.8
33.6
33.4
33.2
33.0
32.8
32.6
6431.6
4292.5
12862
3000 4000
6000
8000 10000
14000
18000
Mass (m/z)

**FIG 1.6**

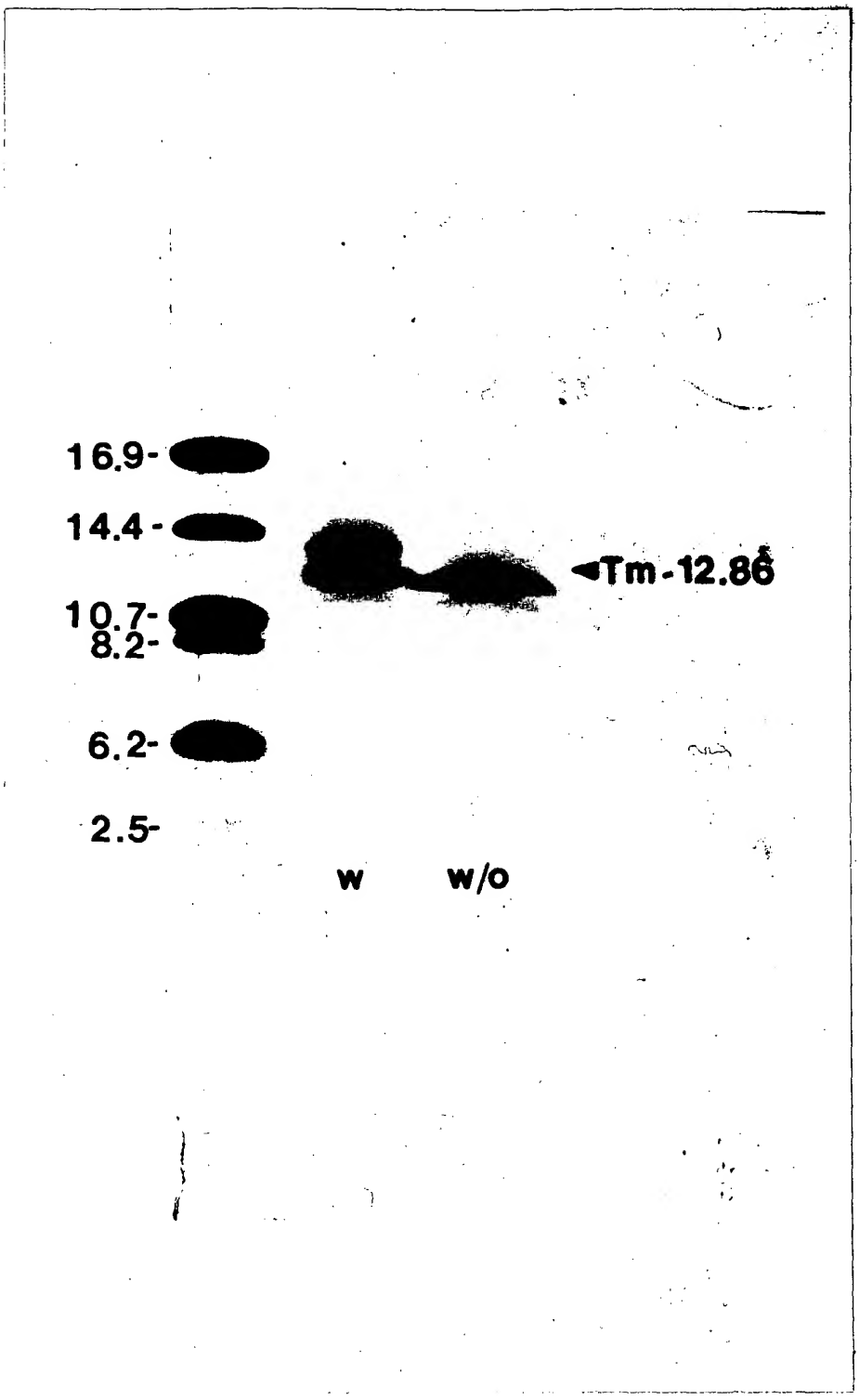



FIG 1.7

**FIG. 1.8**

EP 0 960 945 A1

TO2090 84E92860

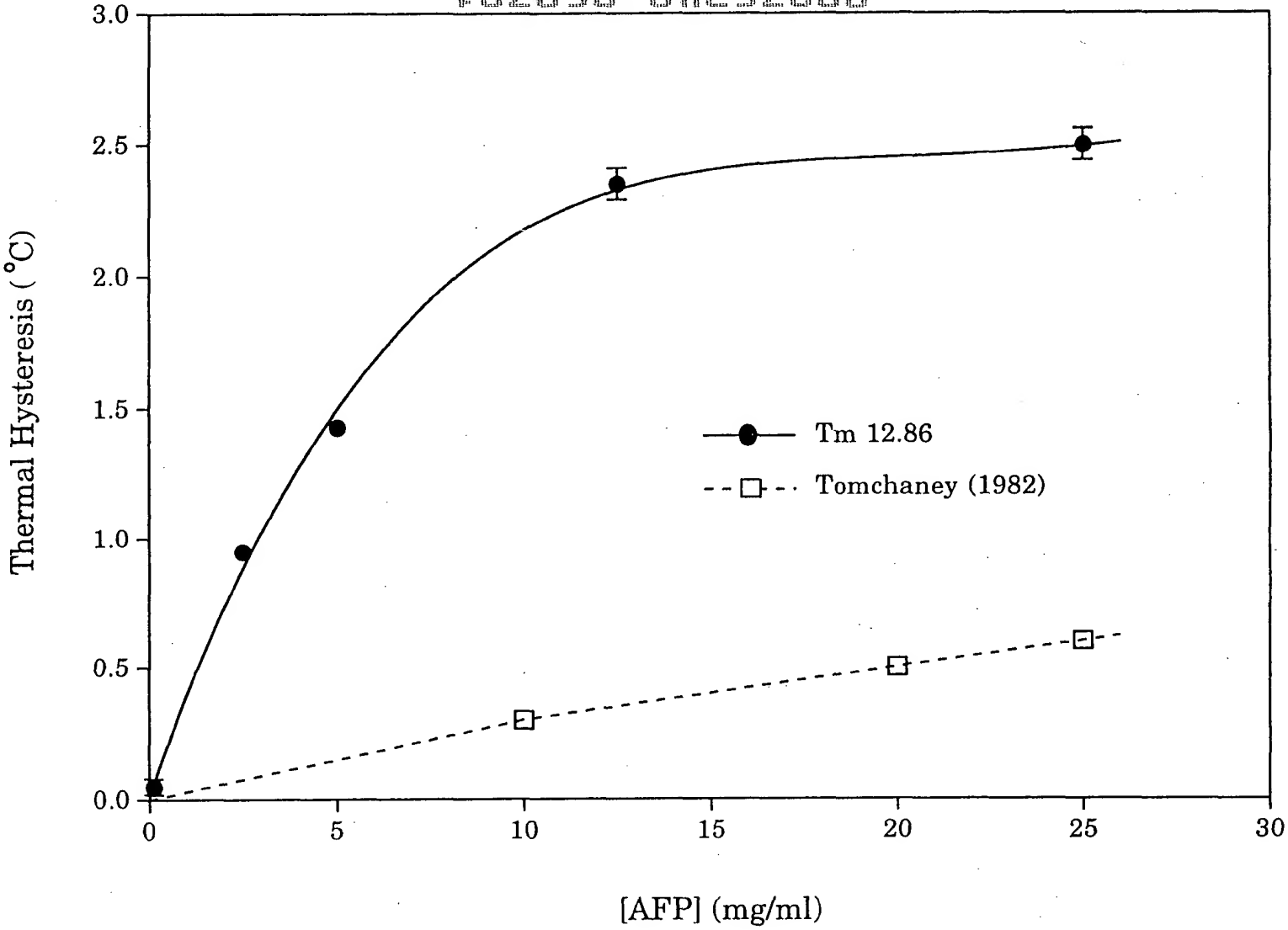



**FIG 1.9**

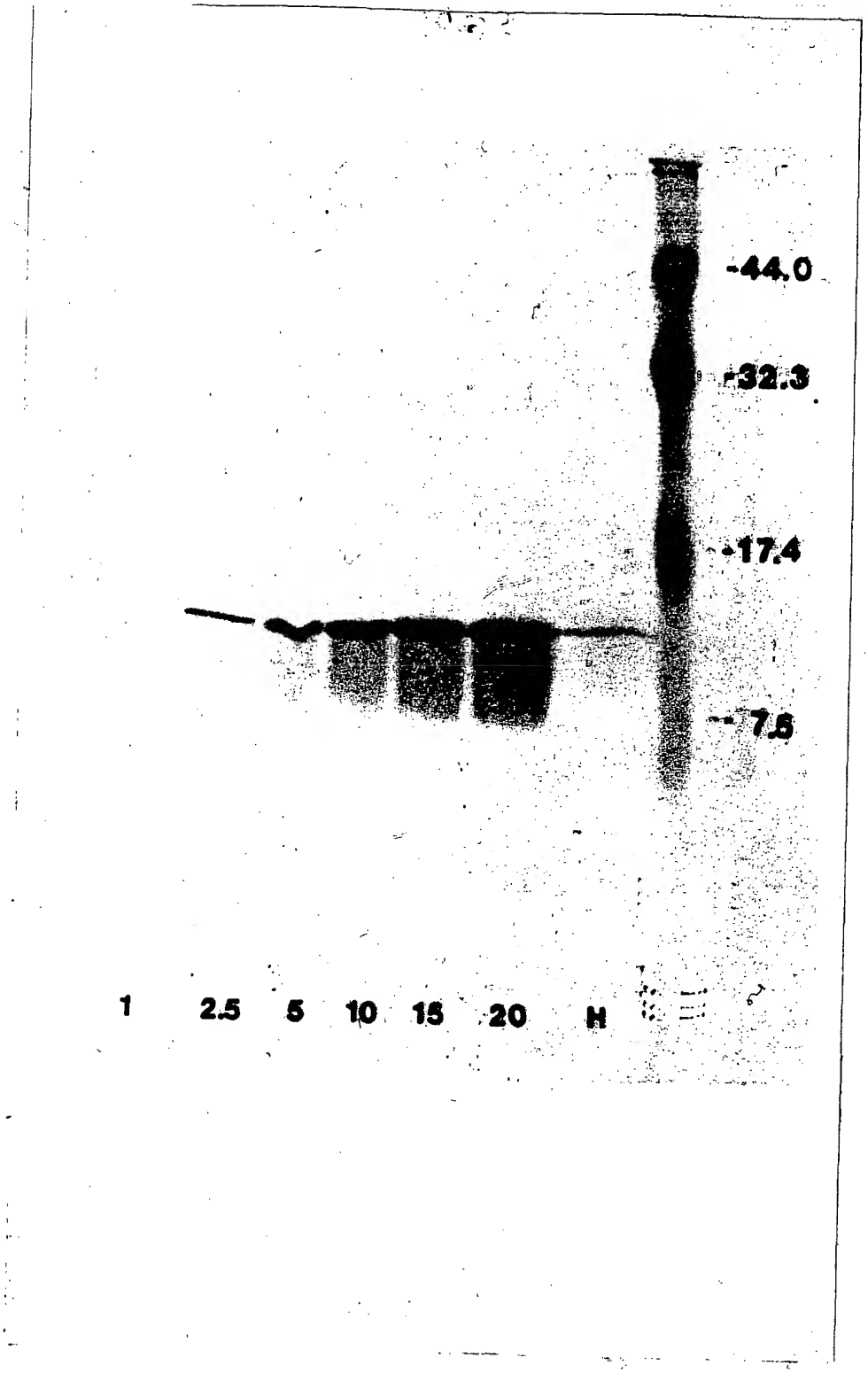



FIG 1.10

TO2090 8hE9Z860

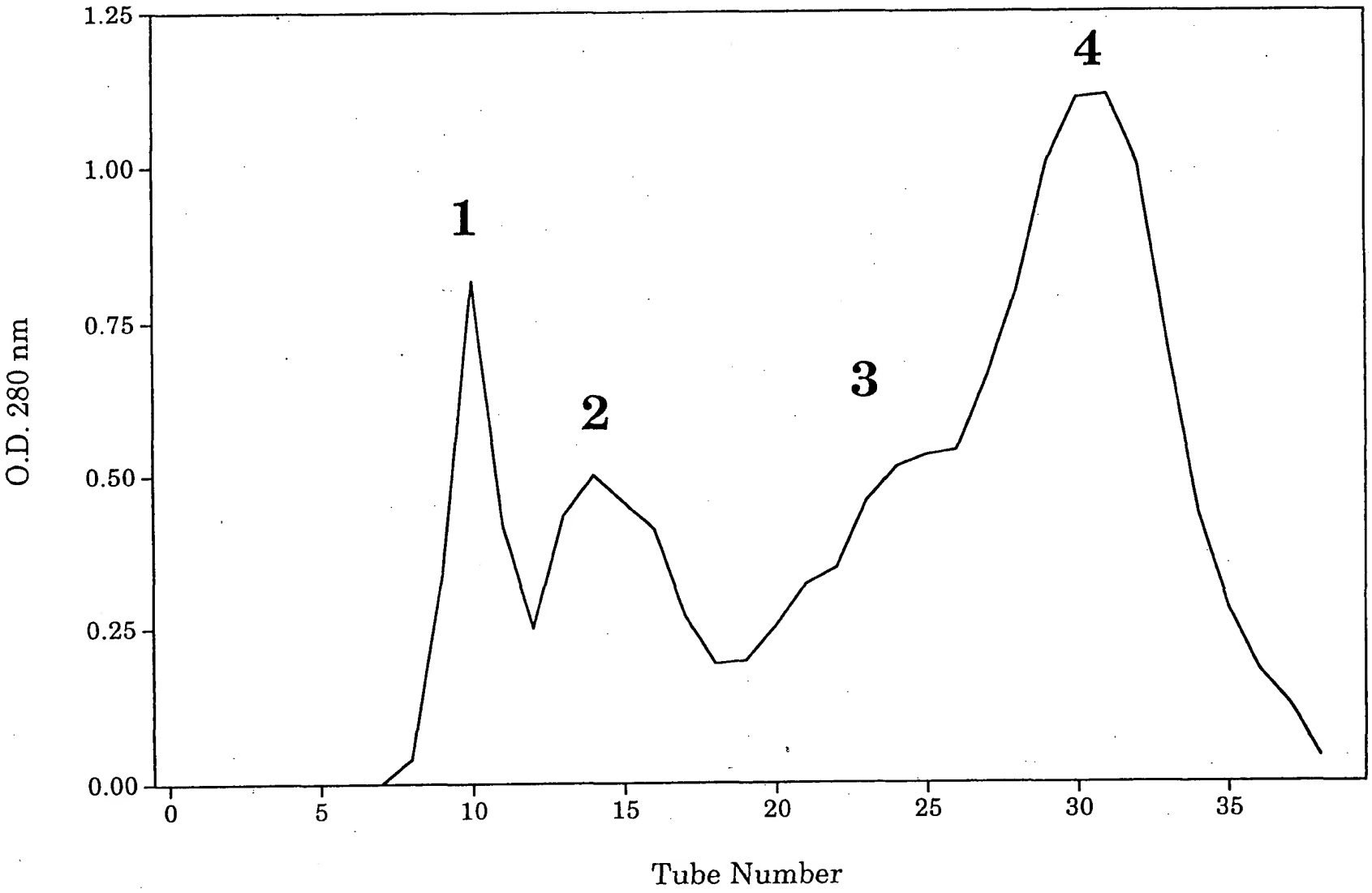



FIG 1.11

WO 90/08860 PCT/US90/00760

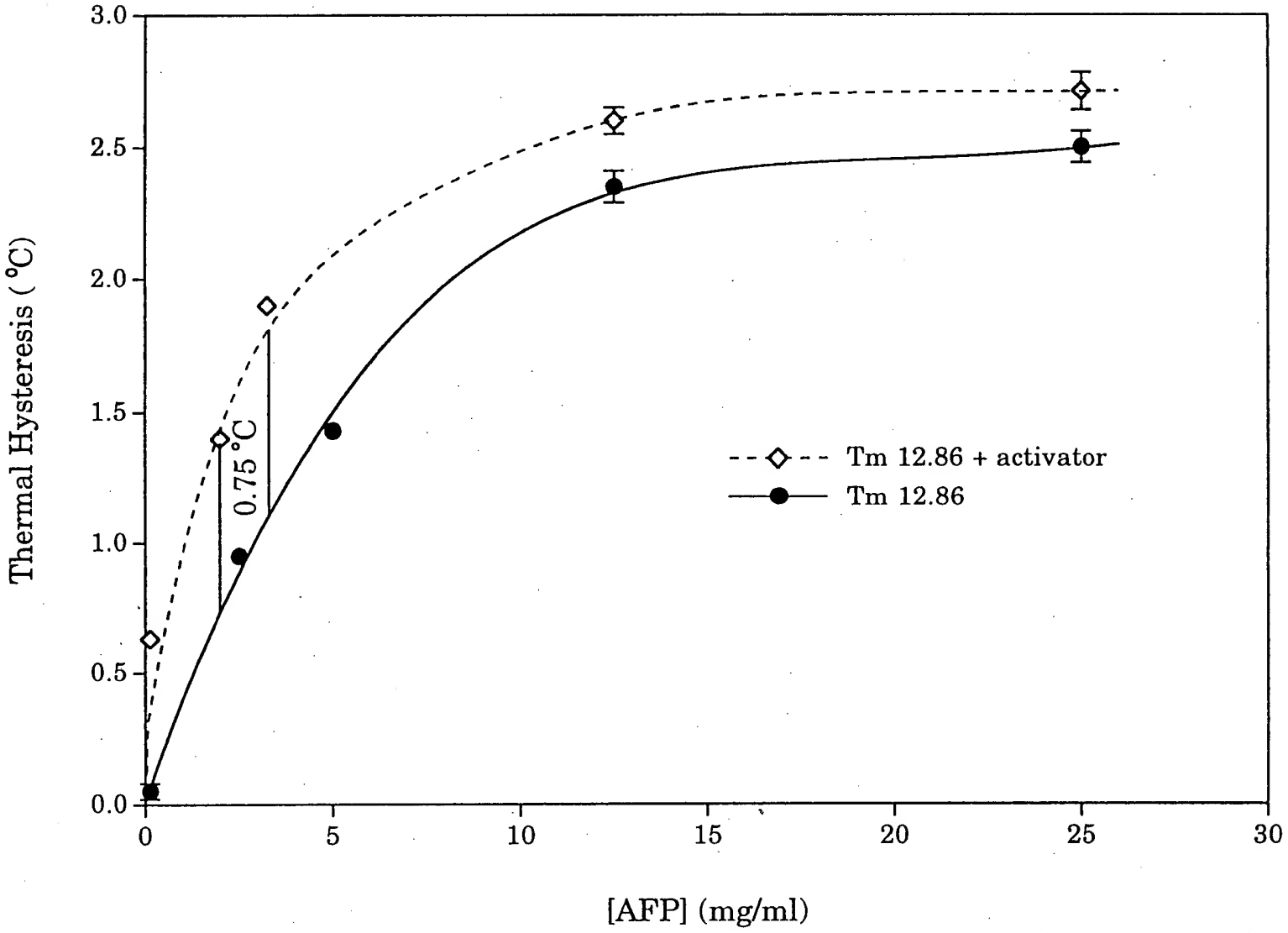



FIG 1.12

PCT/GB94/02650

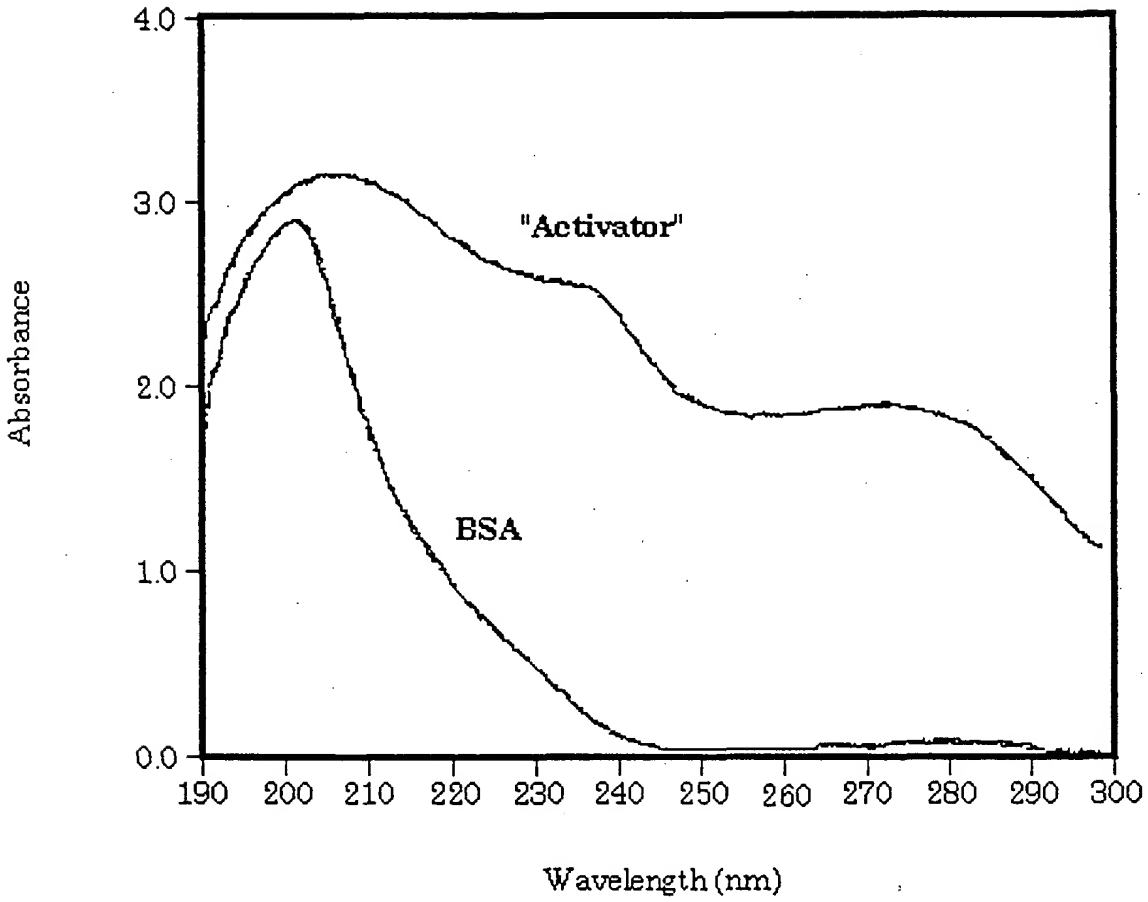



FIG 1.13

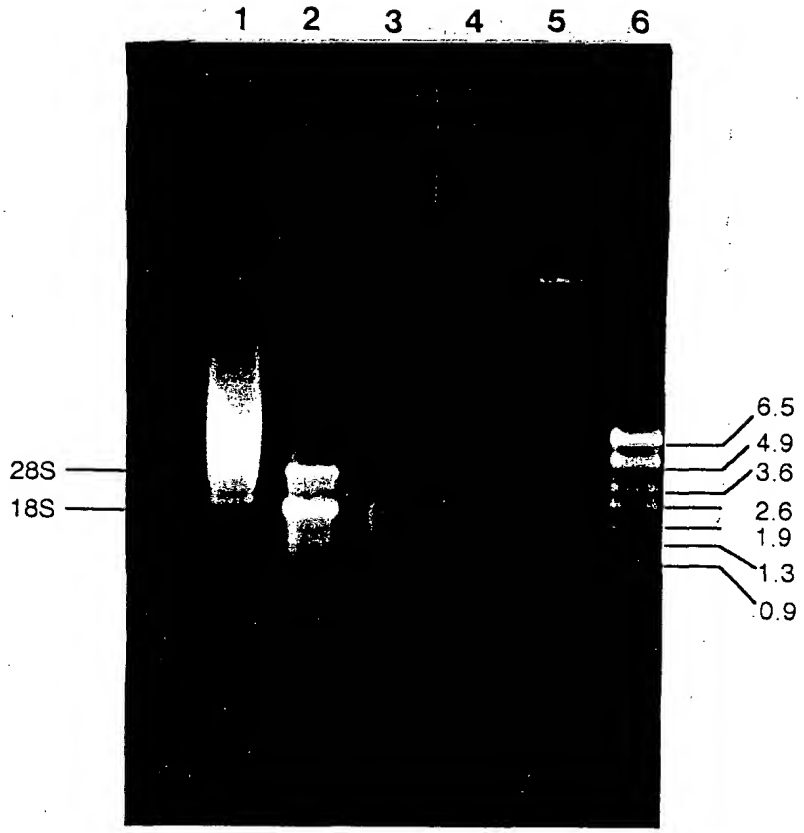



FIG 2.0

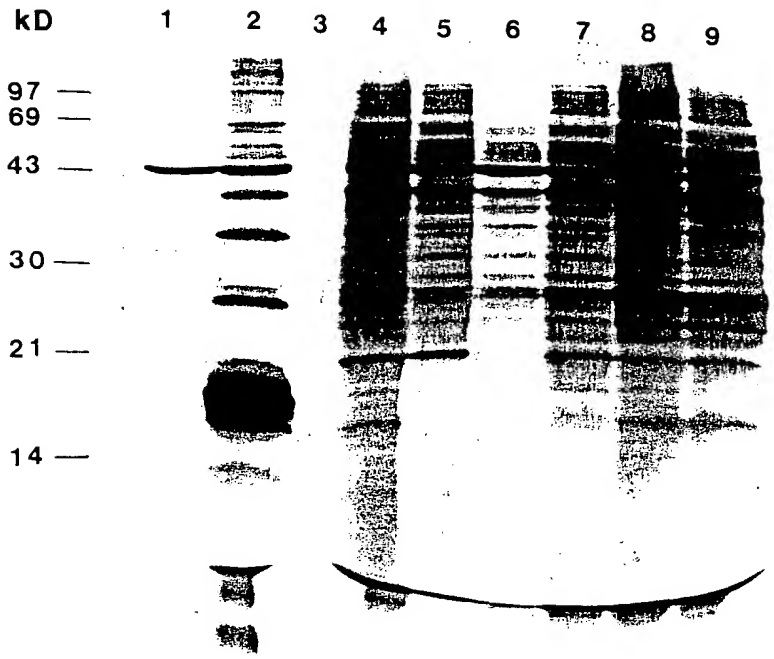



FIG 2.1

EP 0 350 846 A2

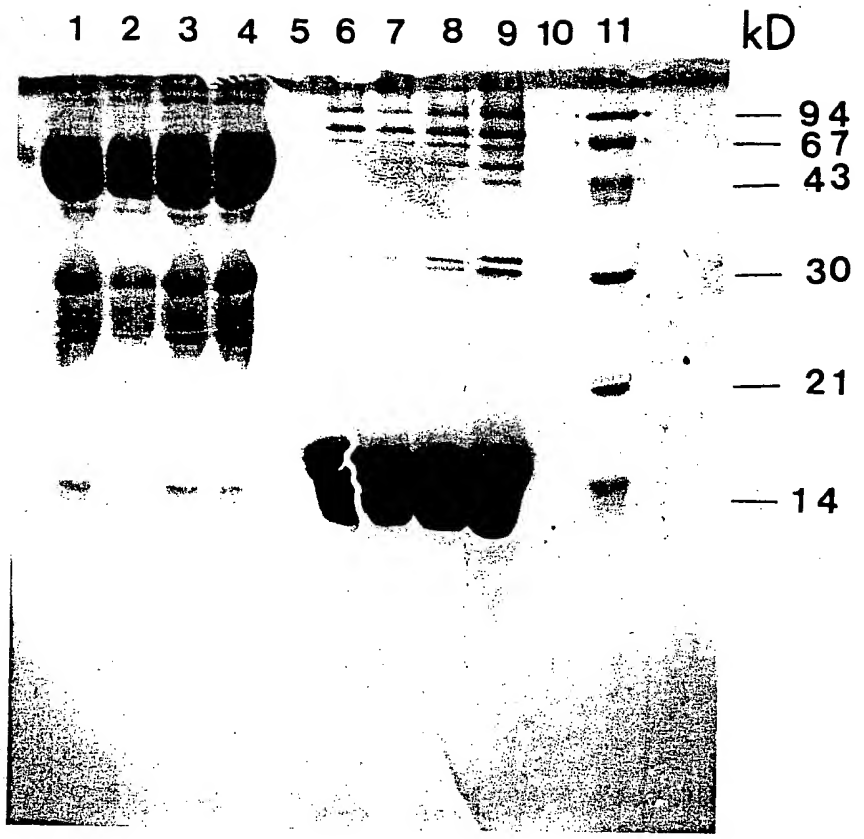



FIG 2.2

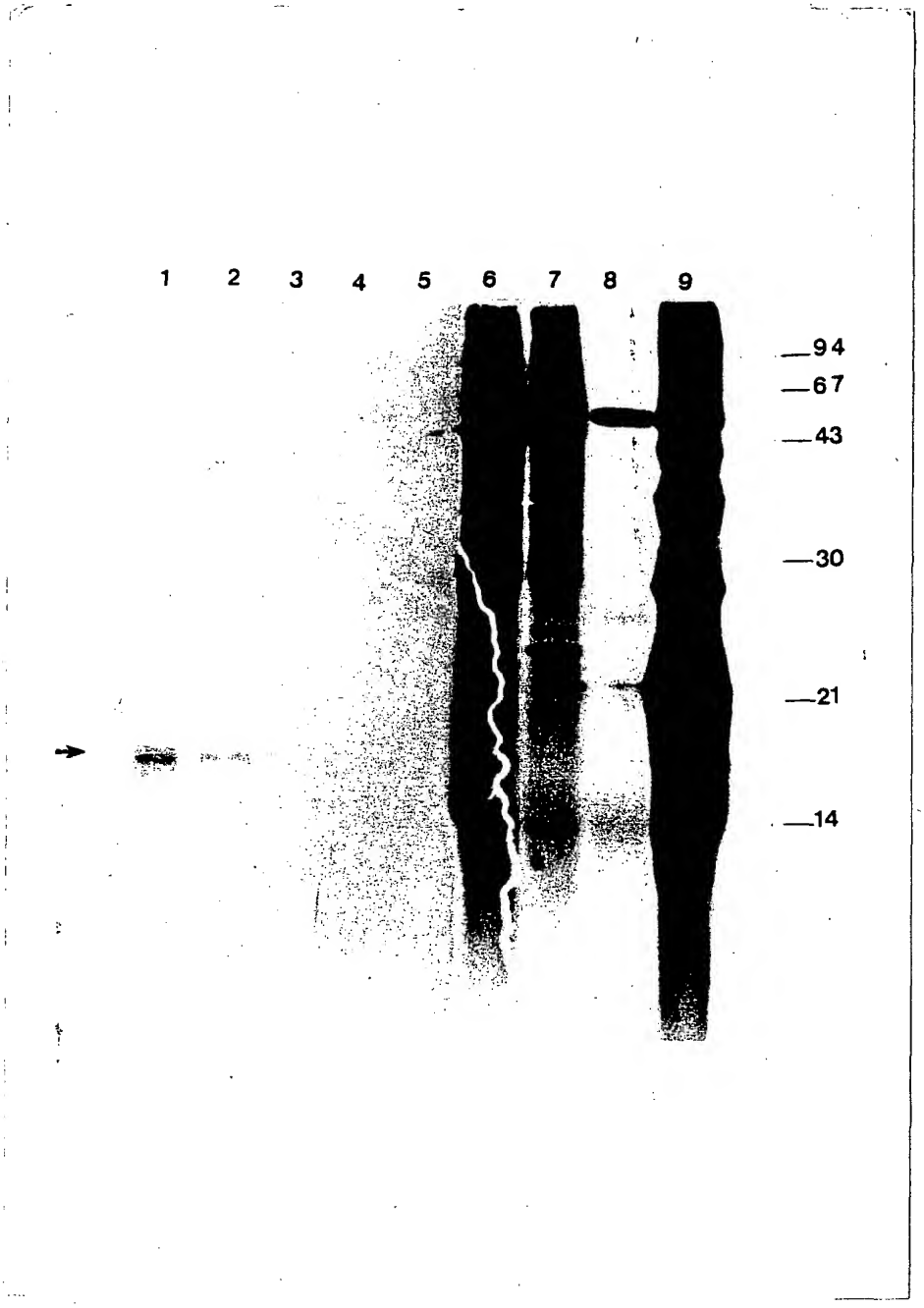



FIG 2.3

PCT/US95/04960

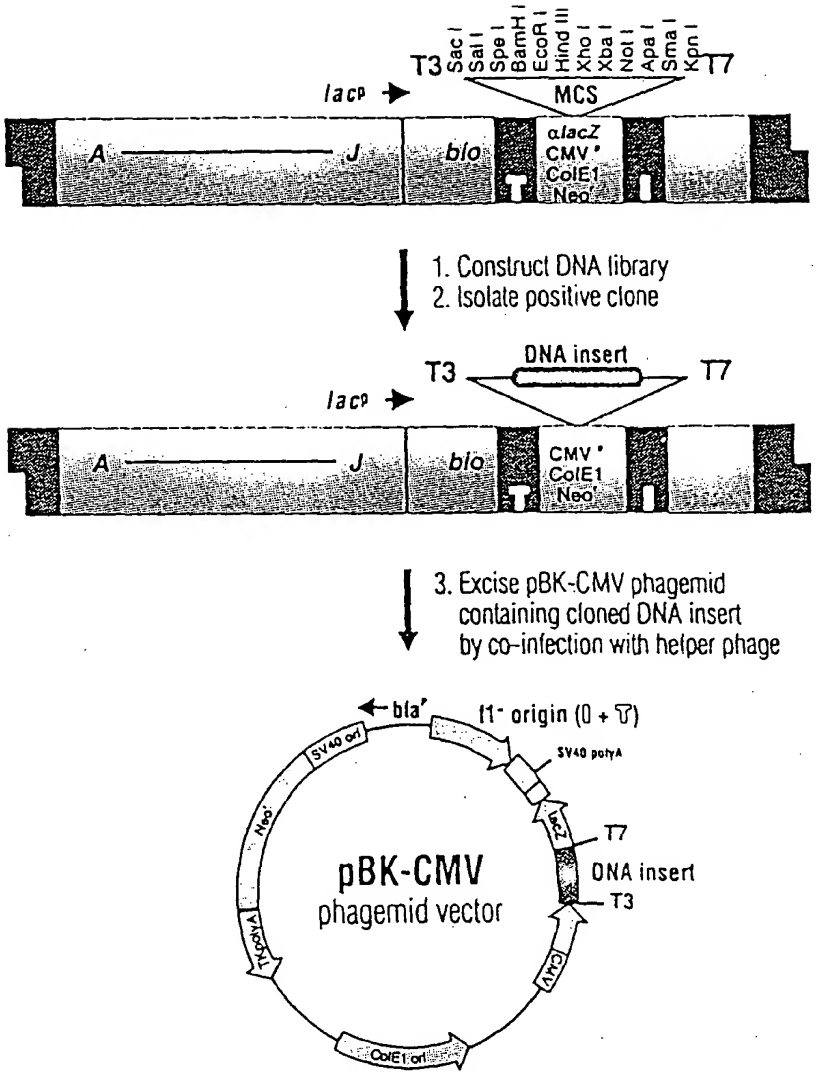
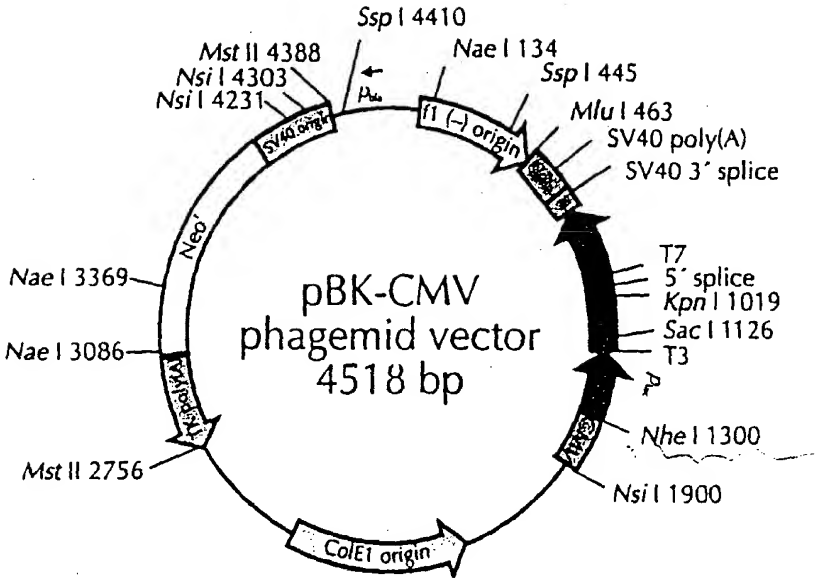



**FIG. 2.4 a**

pBK-CMV phagemid vector



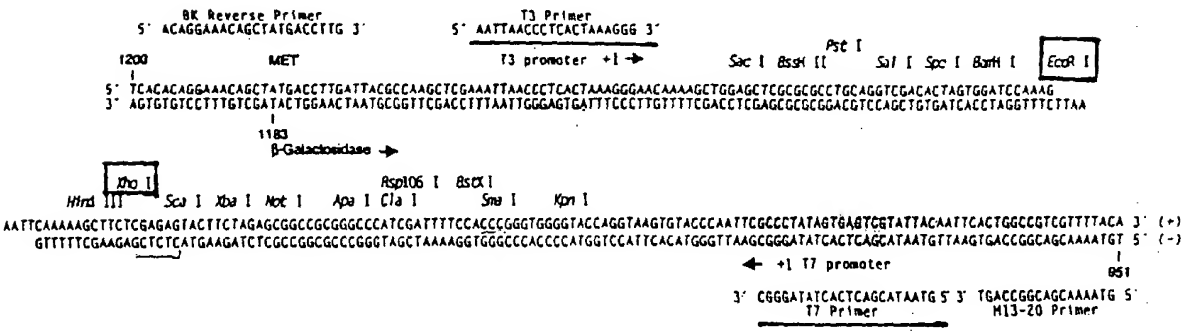




**FIG. 2.4 b**

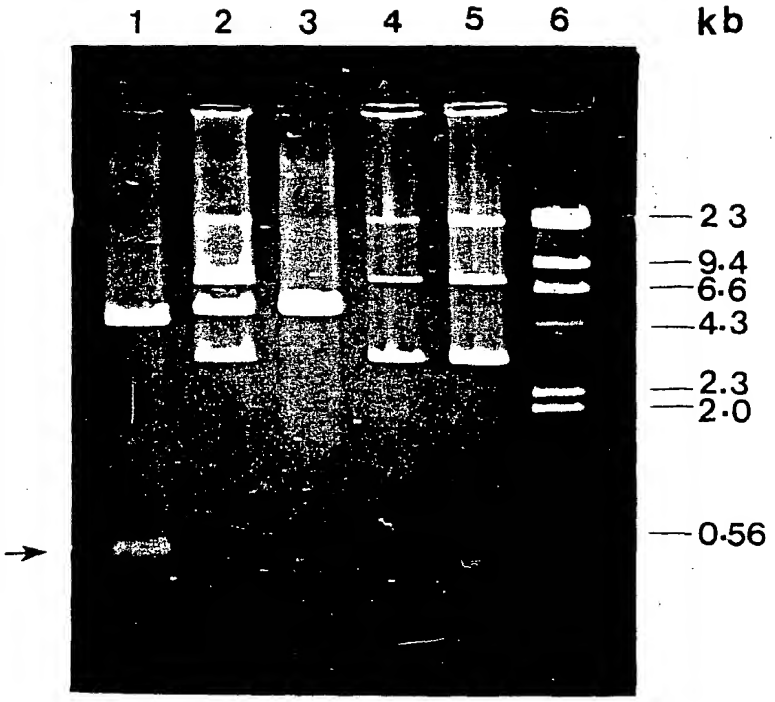



FIG 2.5

# DNA sequence of Tm 13.17 cDNA clone

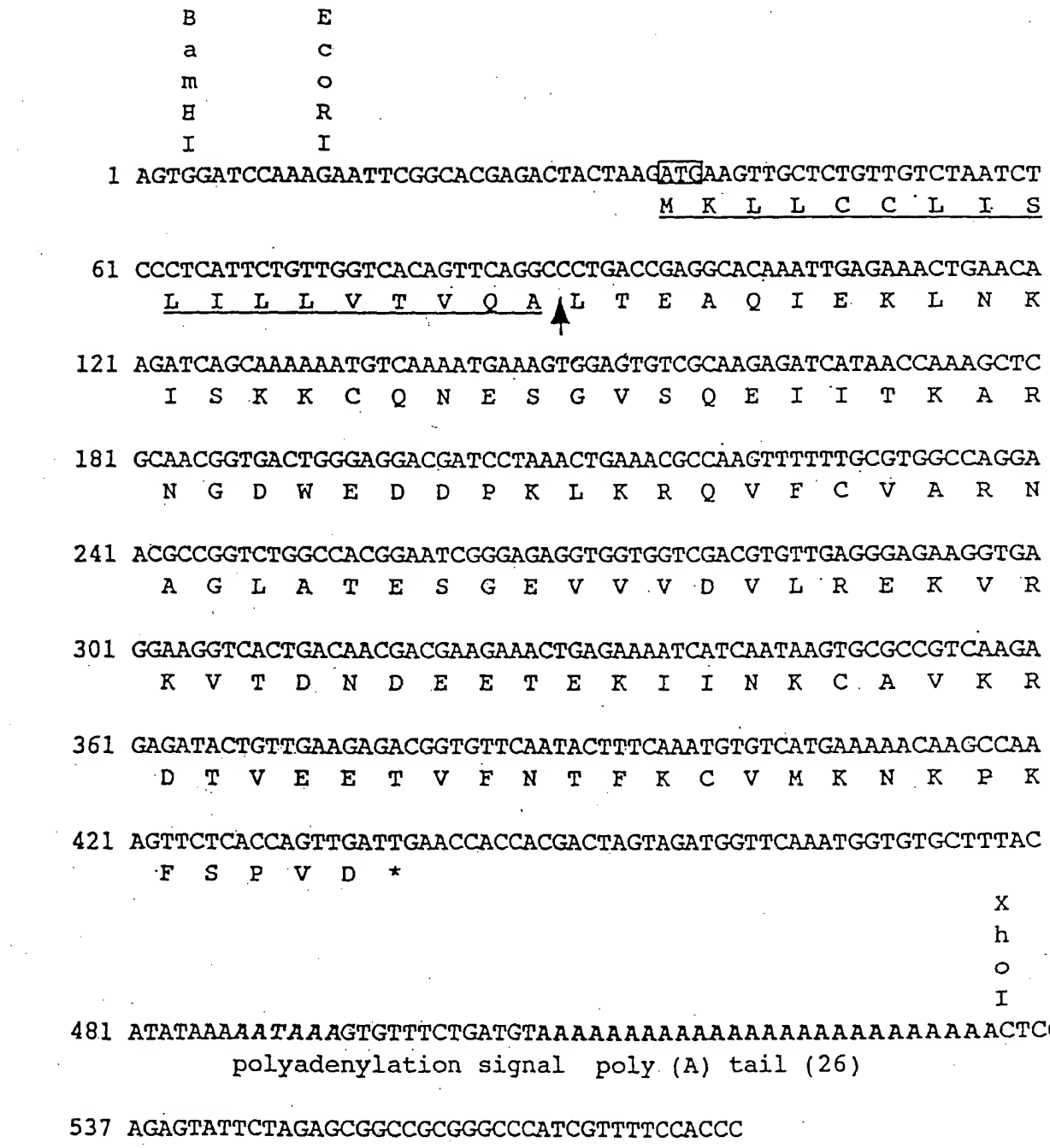



FIG 2.6a

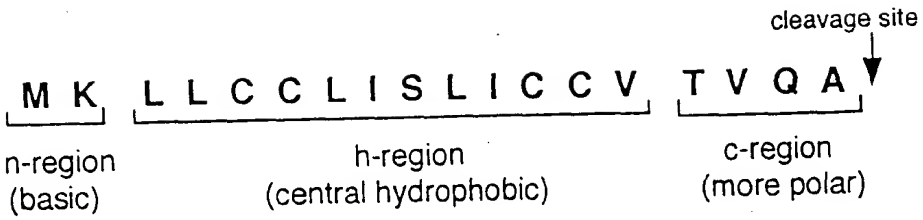

cleavage site
M K
L L C C L I S L I C C V
T V Q A
n-region
(basic)
h-region
(central hydrophobic)
c-region
(more polar)

FIG 2.6b

**A. Mature Tm 13.17 amino acid residure**

```
  1  LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR

 51  NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN

101  TFKCVMKNKP KFSPVD
```

**B. Summary of the composition analysis for the mature Tm 13.17 sequence:**

| Residue | Number | Mole Percent |
|---|---|---|
| A = Ala | 6 | 5.172 |
| B = Asx | 0 | 0.000 |
| C = Cys | 4 | 3.448 |
| D = Asp | 8 | 6.897 |
| E = **Glu** | **13** | **11.207** |
| F = Phe | 4 | 3.448 |
| G = Gly | 4 | 3.448 |
| H = His | 0 | 0.000 |
| I = Ile | 6 | 5.172 |
| K =**Lys** | **16** | **13.793** |
| L = Leu | 5 | 4.310 |
| M = Met | 1 | 0.862 |
| N = Asn | 8 | 6.897 |
| P = Pro | 3 | 2.586 |
| Q = Gln | 4 | 3.448 |
| R = Arg | 6 | 5.172 |
| S = Ser | 5 | 4.310 |
| T = Thr | 8 | 6.897 |
| V =**Val** | **14** | **12.069** |
| W = Trp | 1 | 0.862 |
| Y = Tyr | 0 | 0.000 |
| Z = Glx | 0 | 0.000 |

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

**FIG 2.6c**

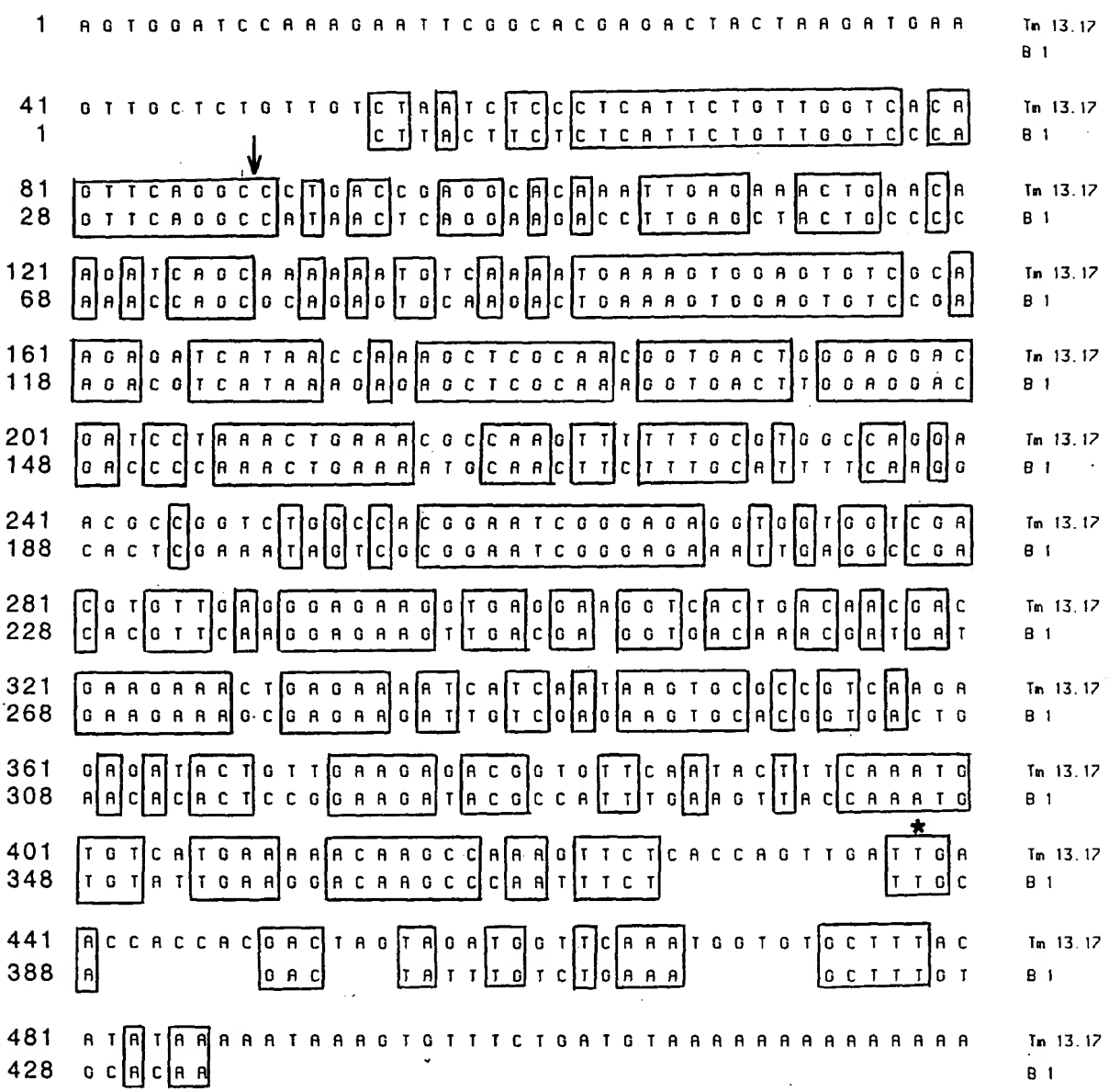

1 AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAA Tn 13.17 B1
41 GTTGCTCTGTTGTCTAATCTCCCTCATTCTGTTGGTCACA Tn 13.17
1 CTTACTTCTCTCATTCTGTTGGTCCCA B1
81 GTTCAGGCCCTGACCGAGGCACAAATTGAGAACTGAACA Tn 13.17
28 GTTCAGGCCAATAACTCAGGAAGACCTTGAGCTACTGCCC B1
121 AGATCAGCAAAAAATGTCAAAATGAAAGTGGAGTGTCGCA Tn 13.17
68 AAACCAGCGCAGAGTGCAAAGACTGAAAGTGGAGTGTCCGA B1
161 AGAGATCATAACCAAAGCTCGCAACGGTGACTGGGAGGAC Tn 13.17
118 AGACGTCATAAAGAGCTCGCAAAGGTGACTTGGAGGAC B1
201 GATCCTAAACTGAAACGCCAAGTTTTTGCGTGGCCAGGA Tn 13.17
148 GACCCCAAACTGAAAATGCAACTTCTTTGCATTTTCAAGGG B1
241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGTGGTGGTCGA Tn 13.17
188 CACTCGAAATAGTCGCGGAATCGGGAGAAATTGAGGCCGA B1
281 CGTGTTGAGGGAGAAGGTGAGGAAGGTCACTGACAACGAC Tn 13.17
228 CACGTTCAAGGAGAAGTTGACGAGGTGACAAACGATGAT B1
321 GAAGAAACTGAGAAAATCATCAATAAGTGCGCCGTCAAGA Tn 13.17
268 GAAGAAAGCGAGAAGATTGTCGAGAAGTGCACGGTGACTG B1
361 GAGATACTGTTGAAGAGACGGTGTTCAAATACTTTCAAATG Tn 13.17
308 AACACACTCCGGAAGATACGCCATTTGAAGTTACCAAATG B1
401 TGTCATGAAAAACAAGCCAAAGTTCTCACCAGTTGATTGA Tn 13.17
348 TGTATTGAAGGACAAGCCCAATTCT TTGC B1
441 ACCACCACGACTAGTAGATGGTTCAAATGGTGTGCTTTAC Tn 13.17
388 A GAC TATTTGTCTGAAA GCTTTGT B1
481 ATATAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAAA Tn 13.17
428 GCACAA B1

FIG 2.7

```
Tm 13.17      3  EAQIEKLNKISKKCQNESGVSQEIITKARNGDWEDDPKLKRQVFCVARNA 52
                 |.. |||.. |. |..|||||:| :.|.|| :  |||||| :.||: :.|
AFP-3         1  ETPREKLKQHSDACKAESGVSEESLNKVRNREEVDDPKLKEHAFCILKRA 50

Tm 13.17     53  GLATESGEVVVDVLREKVRKVTDNDEETEKIINKCAVKRDTVEETVFNTF 102
                 |: ..|||. :| ::.|.:. .::.|..:.::.|||||:||.:..  : |
AFP-3        51  GFIDASGEFQLDHIKTKFKENSEHPEKVDDLVAKCAVKKDTPQHSSADFF 100

Tm 13.17    103  KCVMKNKP 110
                 ||| .|:.
AFP-3       101  KCVHDNRS 108
```

Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

**FIG 2.8**

EP 2 960 264 A1

PCT/US96/05926

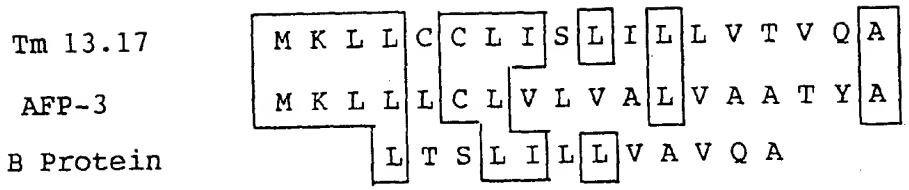



FIG 2.9

EXHIBIT 2136

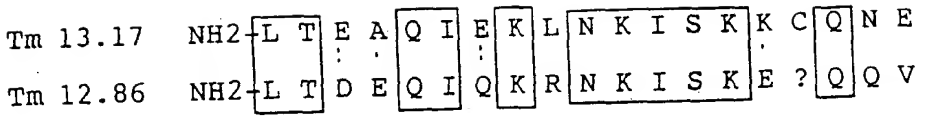



FIG 2.10

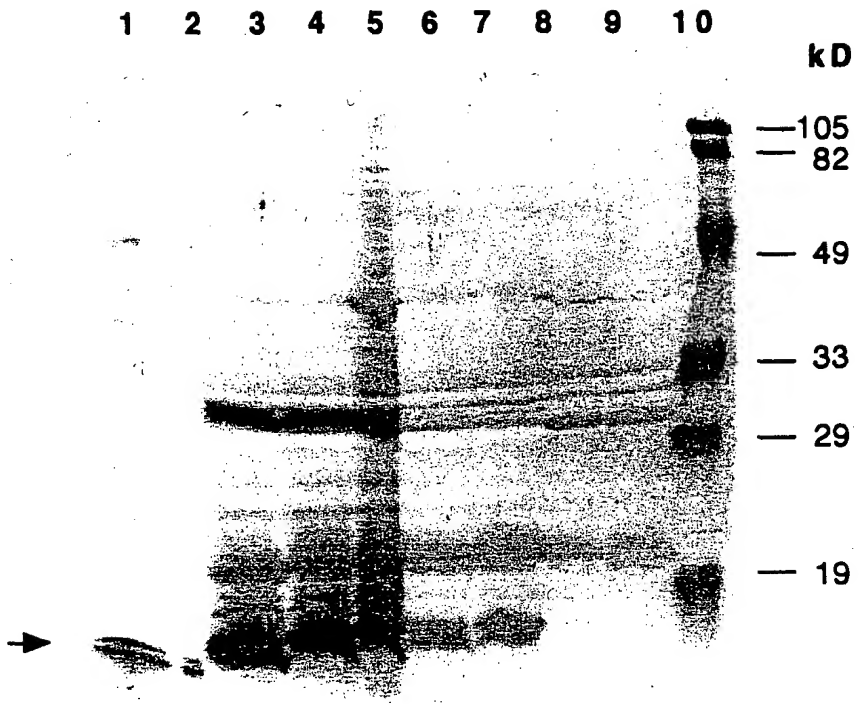



FIG 2.11

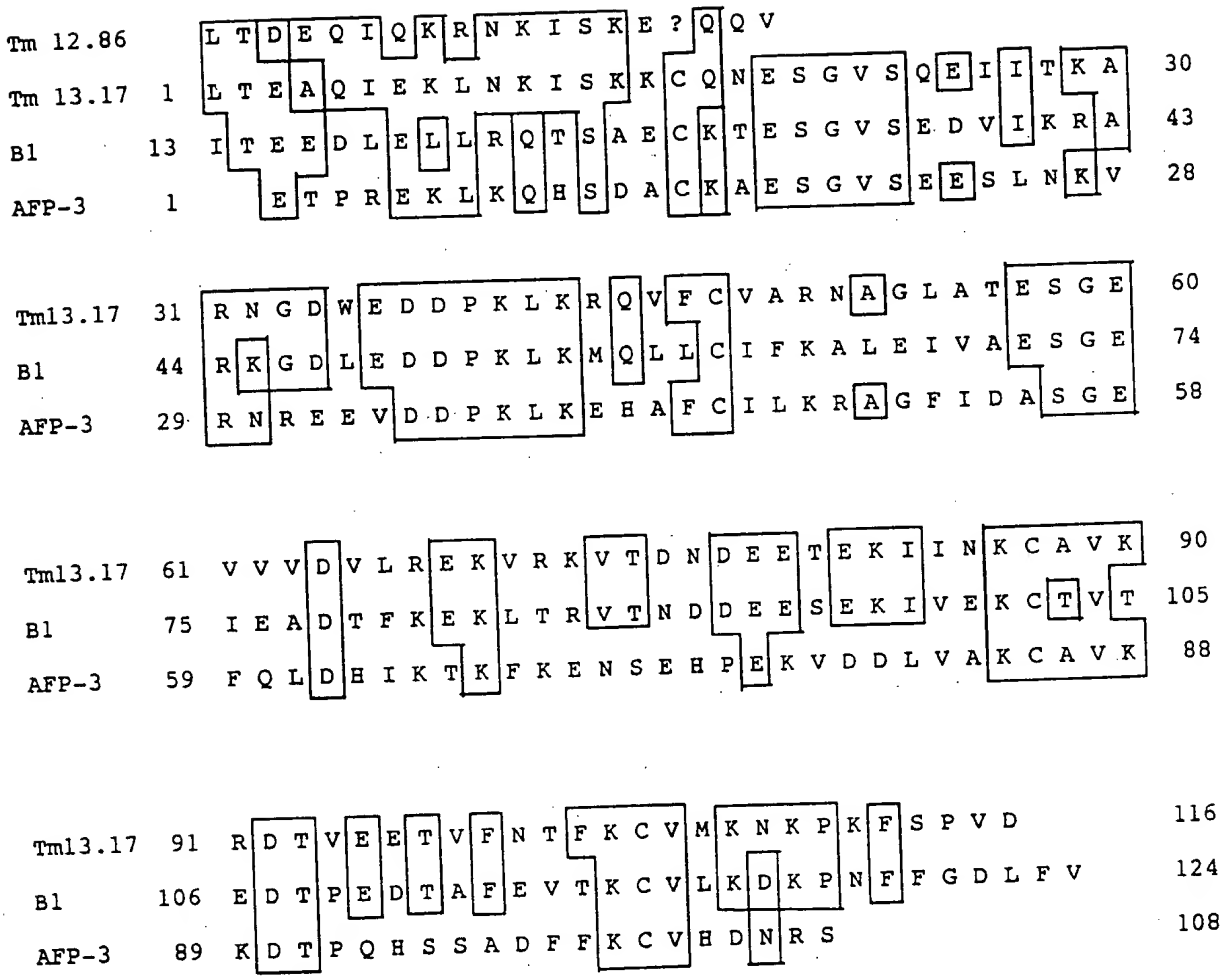
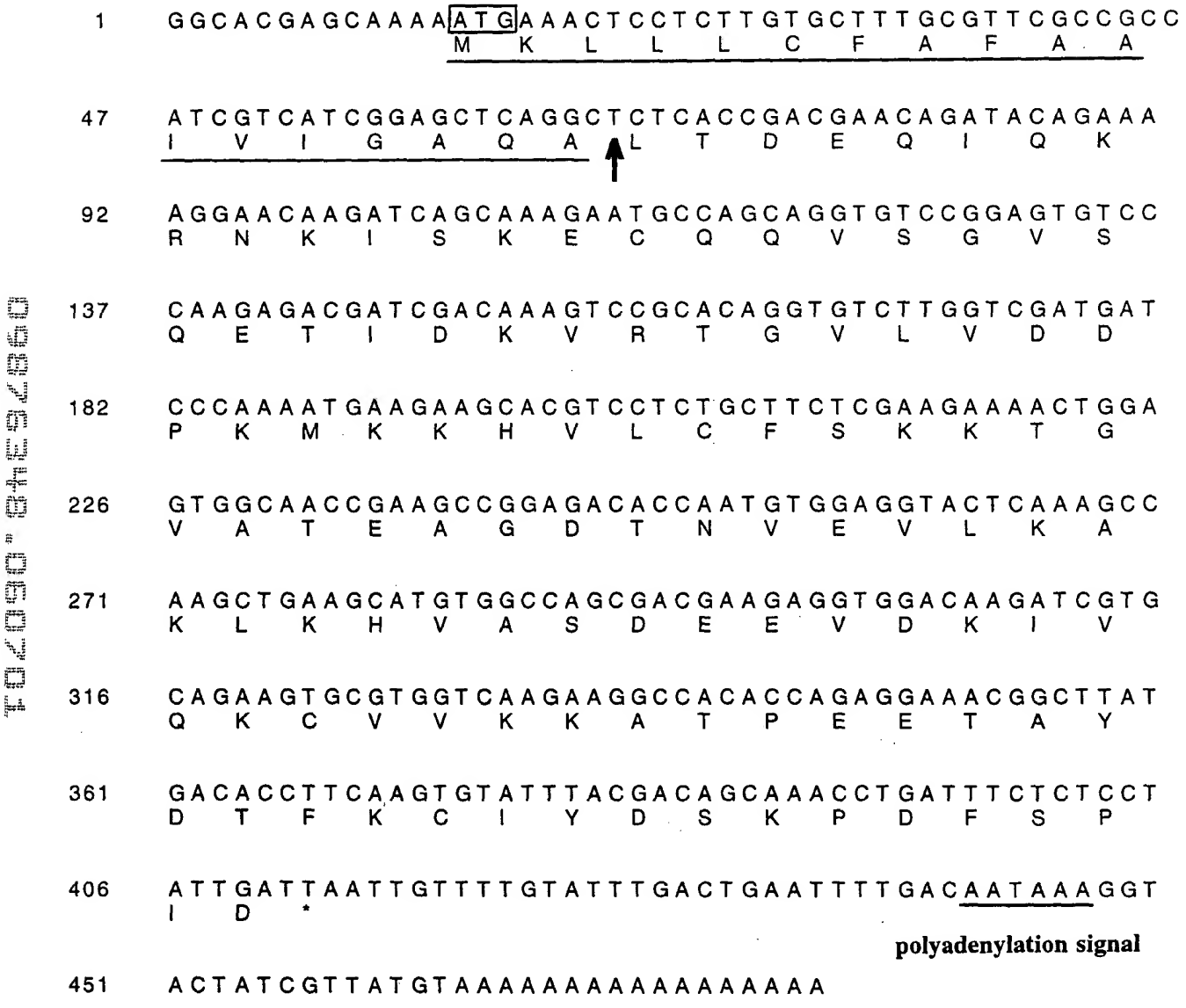

Tm 12.86
Tm 13.17 1 L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A 30
B1 13 I T E E D L E L L R Q T S A E C K T E S G V S E D V I K R A 43
AFP-3 1 E T P R E K L K Q H S D A C K A E S G V S E E S L N K V 28
Tm13.17 31 R N G D W E D D P K L K R Q V F C V A R N A G L A T E S G E 60
B1 44 R K G D L E D D P K L K M Q L L C I F K A L E I V A E S G E 74
AFP-3 29 R N R E E V D D P K L K E H A F C I L K R A G F I D A S G E 58
Tm13.17 61 V V V D V L R E K V R K V T D N D E E T E K I I N K C A V K 90
B1 75 I E A D T F K E K L T R V T N D D E E S E K I V E K C T V T 105
AFP-3 59 F Q L D H I K T K F K E N S E H P E K V D D L V A K C A V K 88
Tm13.17 91 R D T V E E T V F N T F K C V M K N K P K F S P V D 116
B1 106 E D T P E D T A F E V T K C V L K D K P N F F G D L F V 124
AFP-3 89 K D T P Q H S S A D F F K C V H D N R S 108

FIG 2.12




poly (A) tail

FIG 3.0

DOCKET NO. 50P3645.00

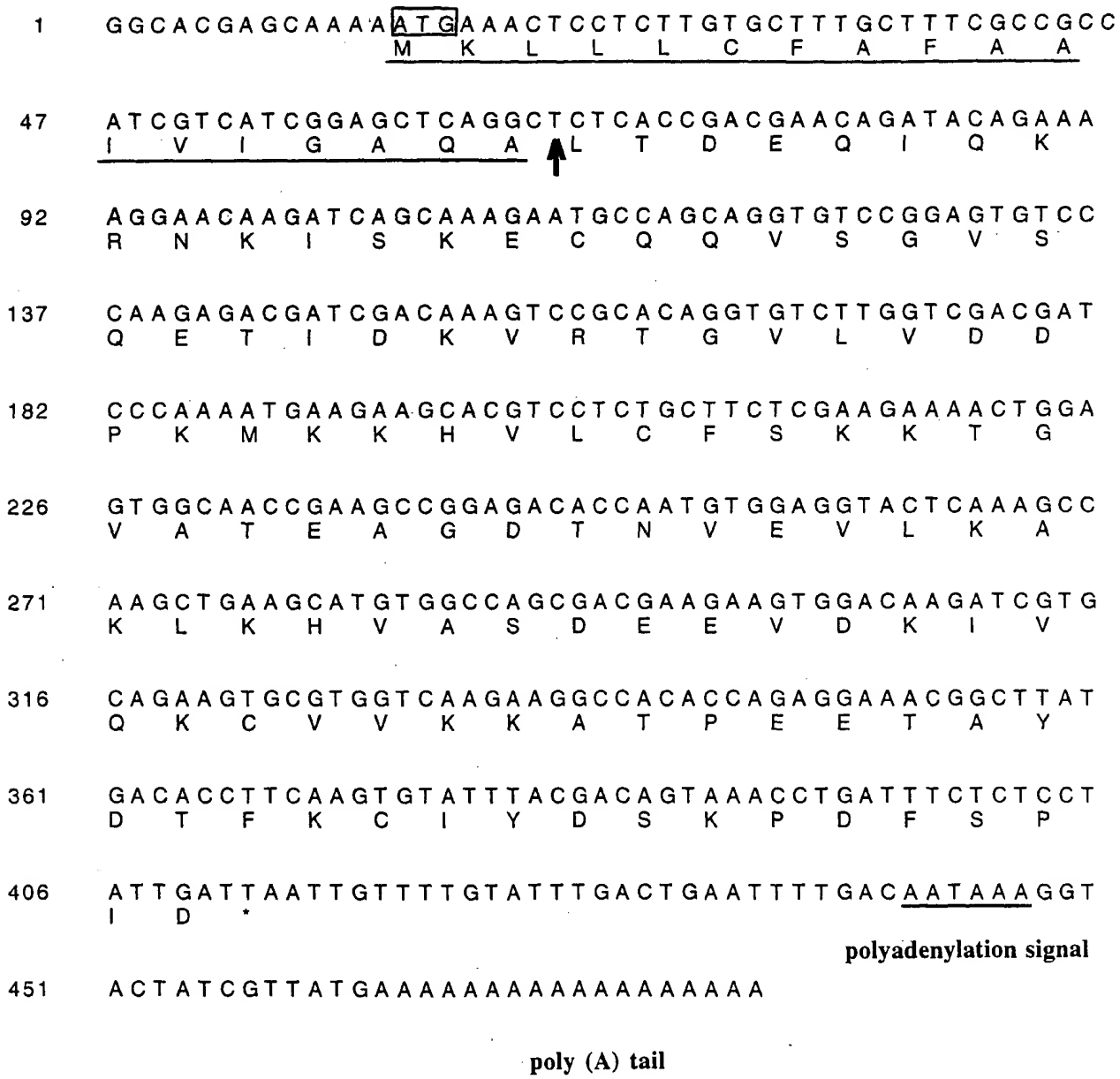



FIG 3.1

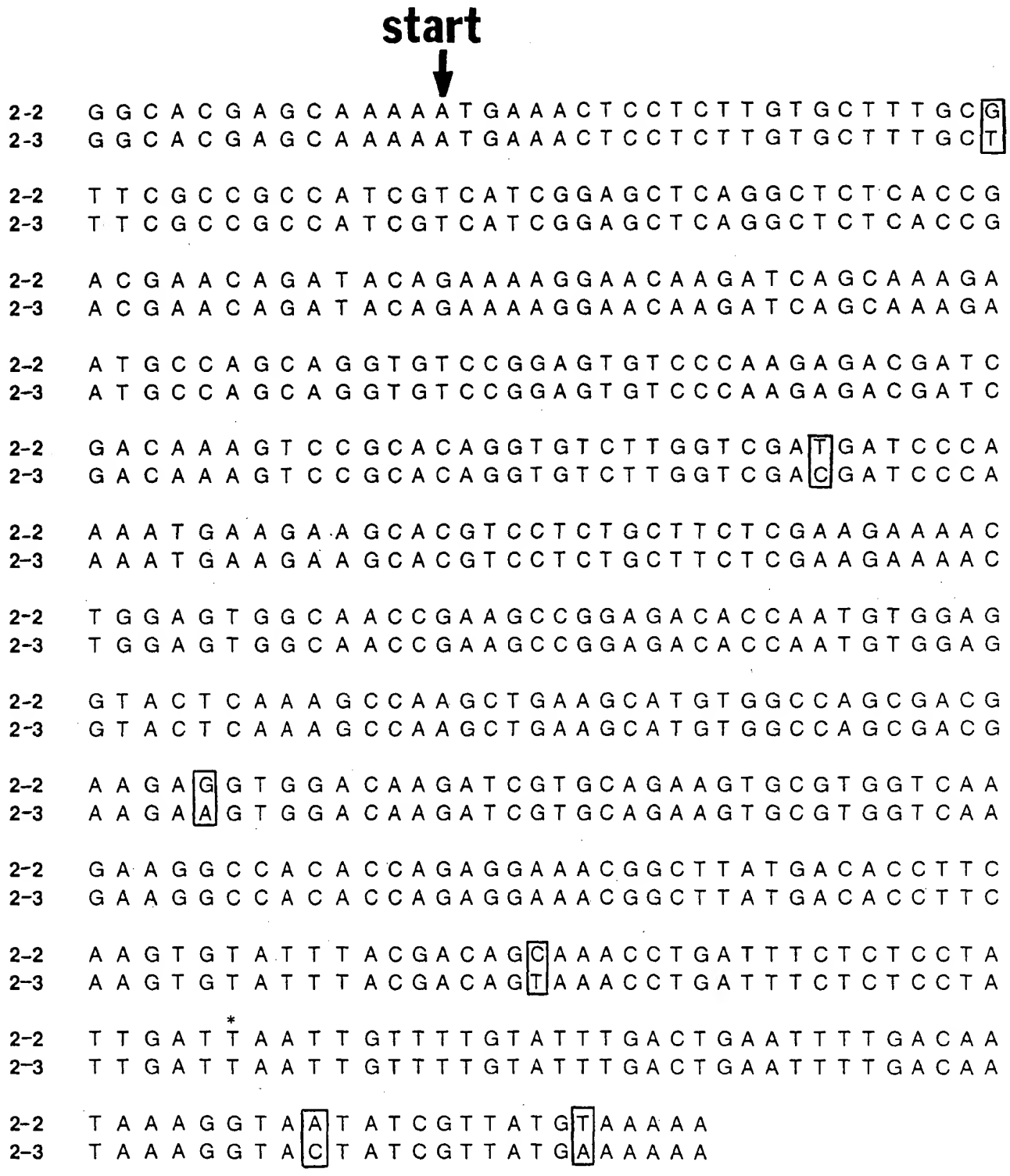

start
2-2 G G C A C G A G C A A A A A T G A A A C T C C T C T T G T G C T T T G C G
2-3 G G C A C G A G C A A A A A T G A A A C T C C T C T T G T G C T T T G C T
2-2 T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G
2-3 T T C G C C G C C A T C G T C A T C G G A G C T C A G G C T C T C A C C G
2-2 A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A
2-3 A C G A A C A G A T A C A G A A A A G G A A C A A G A T C A G C A A A G A
2-2 A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C
2-3 A T G C C A G C A G G T G T C C G G A G T G T C C C A A G A G A C G A T C
2-2 G A C A A A G T C C G C A C A G G T G T C T T G G T C G A T G A T C C C A
2-3 G A C A A A G T C C G C A C A G G T G T C T T G G T C G A C G A T C C C A
2-2 A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A A C
2-3 A A A T G A A G A A G C A C G T C C T C T G C T T C T C G A A G A A A A C
2-2 T G G A G T G G C A A C C G A A G C C G G A G A C A C C A A T G T G G A G
2-3 T G G A G T G G C A A C C G A A G C C G G A G A C A C C A A T G T G G A G
2-2 G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G
2-3 G T A C T C A A A G C C A A G C T G A A G C A T G T G G C C A G C G A C G
2-2 A A G A G G T G G A C A A G A T C G T G C A G A A G T G C G T G G T C A A
2-3 A A G A A G T G G A C A A G A T C G T G C A G A A G T G C G T G G T C A A
2-2 G A A G G C C A C A C C A G A G G A A A C G G C T T A T G A C A C C T T C
2-3 G A A G G C C A C A C C A G A G G A A A C G G C T T A T G A C A C C T T C
2-2 A A G T G T A T T T A C G A C A G C A A A C C T G A T T T C T C T C C T A
2-3 A A G T G T A T T T A C G A C A G T A A A C C T G A T T T C T C T C C T A
2-2 T T G A T T A A T T G T T T T G T A T T T G A C T G A A T T T T G A C A A
2-3 T T G A T T A A T T G T T T T G T A T T T G A C T G A A T T T T G A C A A
2-2 T A A A G G T A A T A T C G T T A T G T A A A A A
2-3 T A A A G G T A C T A T C G T T A T G A A A A A A

FIG. 3.2

Predicted Amino Acid

Composition of 2-2 and 2-3

| Analysis | Whole Protein |
|---|---|
| Molecular Weight | 12843.80 m.w. |
| Length | 115 |
| 1 microgram = | 77.859 pMoles |
| Molar Extinction coefficient | 3040±5% |
| 1 A(280) = | 4.22 mg/ml |
| Isoelectric Point | 7.11 |
| Charge at pH 7 | 0.13 |

Whole Protein Composition Analysis

| Amino Acid(s) | Number count | % by weight | % by frequency |
|---|---|---|---|
| Charged (RKHYCDE) | 48 | 47.19 | 41.74 |
| Acidic (DE) | 20 | 18.90 | 17.39 |
| Basic (KR) | 20 | 20.40 | 17.39 |
| Polar (NCQSTY) | 30 | 25.35 | 26.09 |
| Hydrophobic (AILFWV) | 34 | 27.26 | 29.57 |
| A Ala | 6 | 3.32 | 5.22 |
| C Cys | 4 | 3.21 | 3.48 |
| D Asp | 11 | 9.86 | 9.57 |
| E Glu | 9 | 9.05 | 7.83 |
| F Phe | 3 | 3.44 | 2.61 |
| G Gly | 4 | 1.78 | 3.48 |
| H His | 2 | 2.14 | 1.74 |
| I Ile | 6 | 5.29 | 5.22 |
| K Lys | 18 | 17.97 | 15.65 |
| L Leu | 5 | 4.41 | 4.35 |
| M Met | 1 | 1.02 | 0.87 |
| N Asn | 2 | 1.78 | 1.74 |
| P Pro | 4 | 3.02 | 3.48 |
| Q Gln | 6 | 5.98 | 5.22 |
| R Arg | 2 | 2.43 | 1.74 |
| S Ser | 7 | 4.75 | 6.09 |
| T Thr | 9 | 7.08 | 7.83 |
| V Val | 14 | 10.80 | 12.17 |
| W Trp | 0 | 0.00 | 0.00 |
| Y Tyr | 2 | 2.54 | 1.74 |
| B Asx | 0 | 0.00 | 0.00 |
| Z Glx | 0 | 0.00 | 0.00 |
| X Xxx | 0 | 0.00 | 0.00 |
| . Ter | 0 | 0.00 | 0.00 |

FIG 3.3

09/03/2004 09:09:08

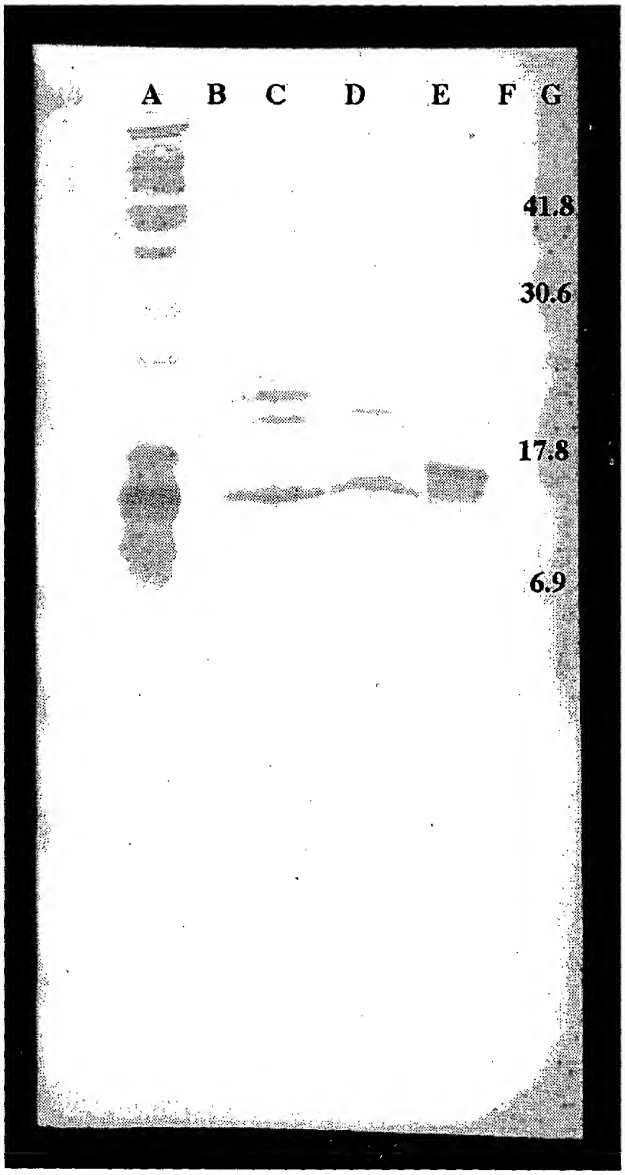



FIG 3.4

EP 0 969 094 A1

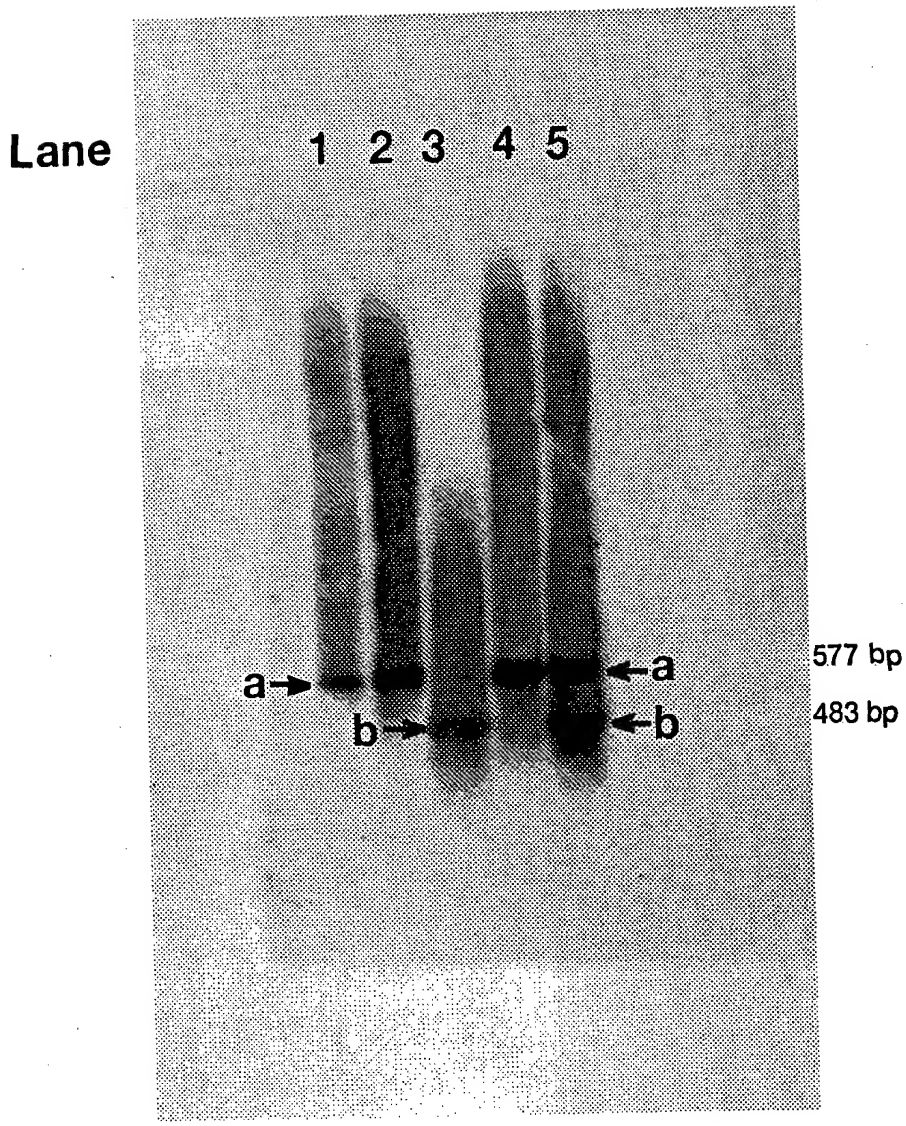



FIG 4.0

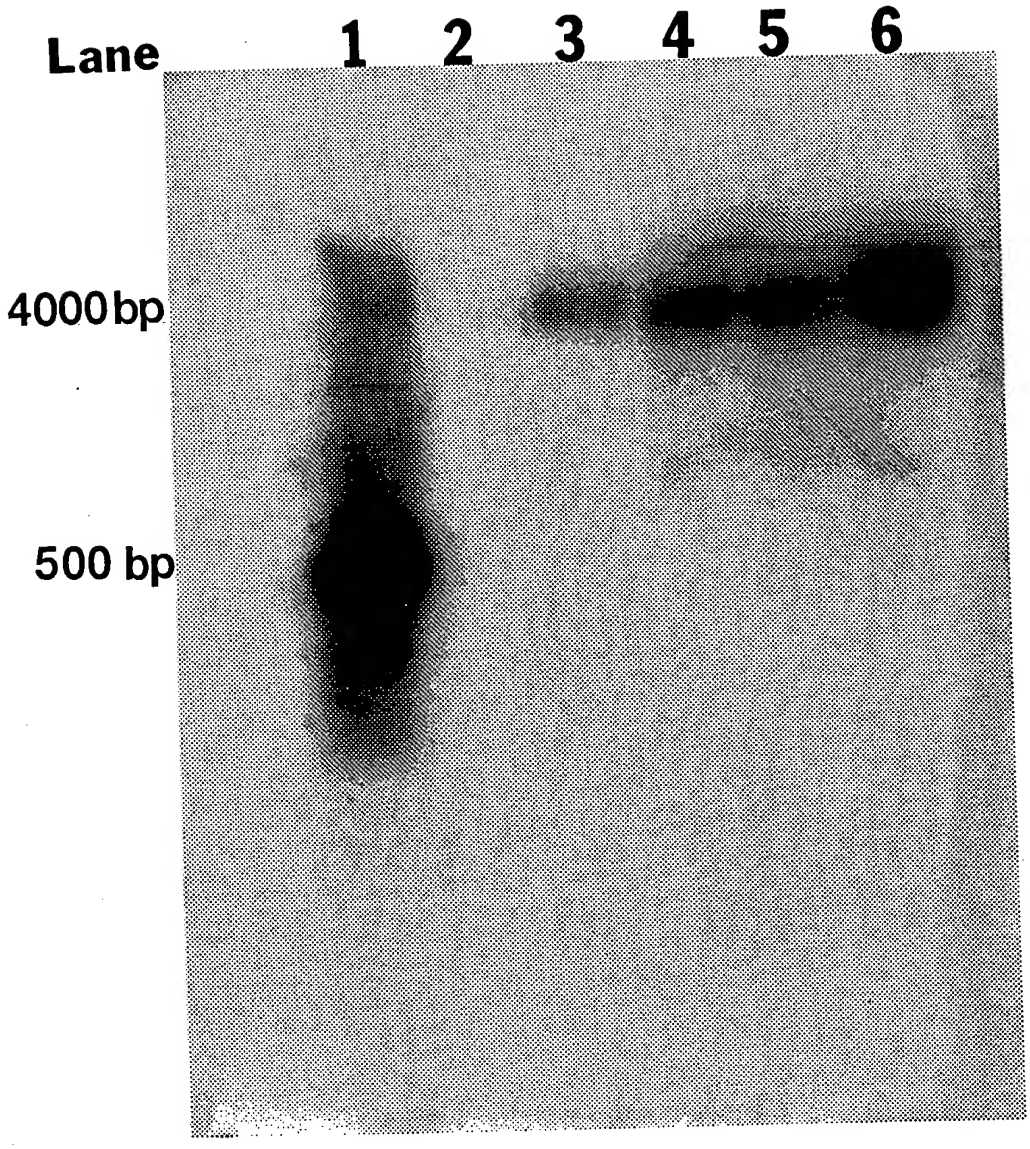



FIG 4.1

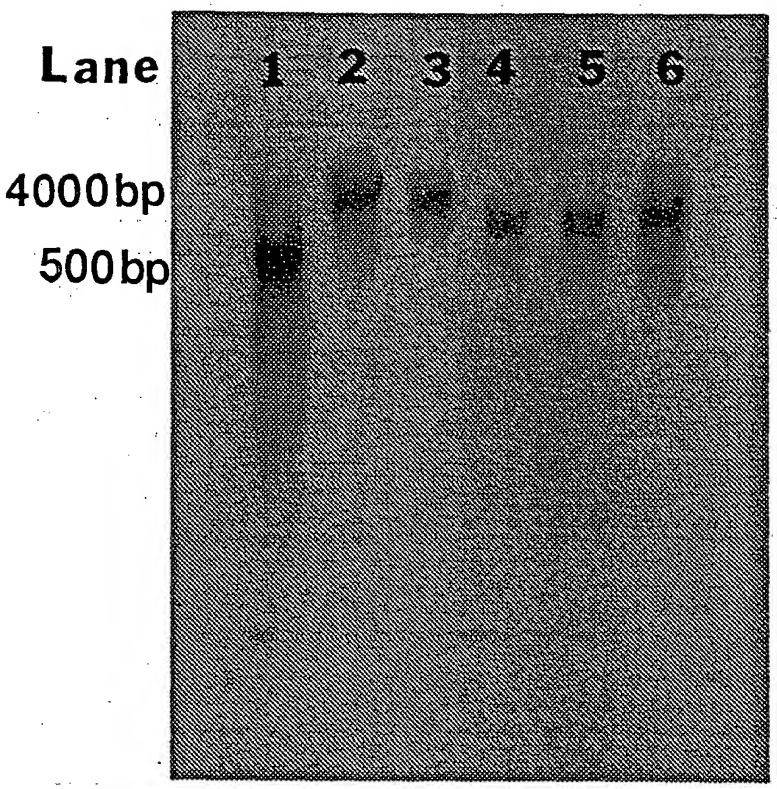



FIG 4.2

TO2090'84E97860

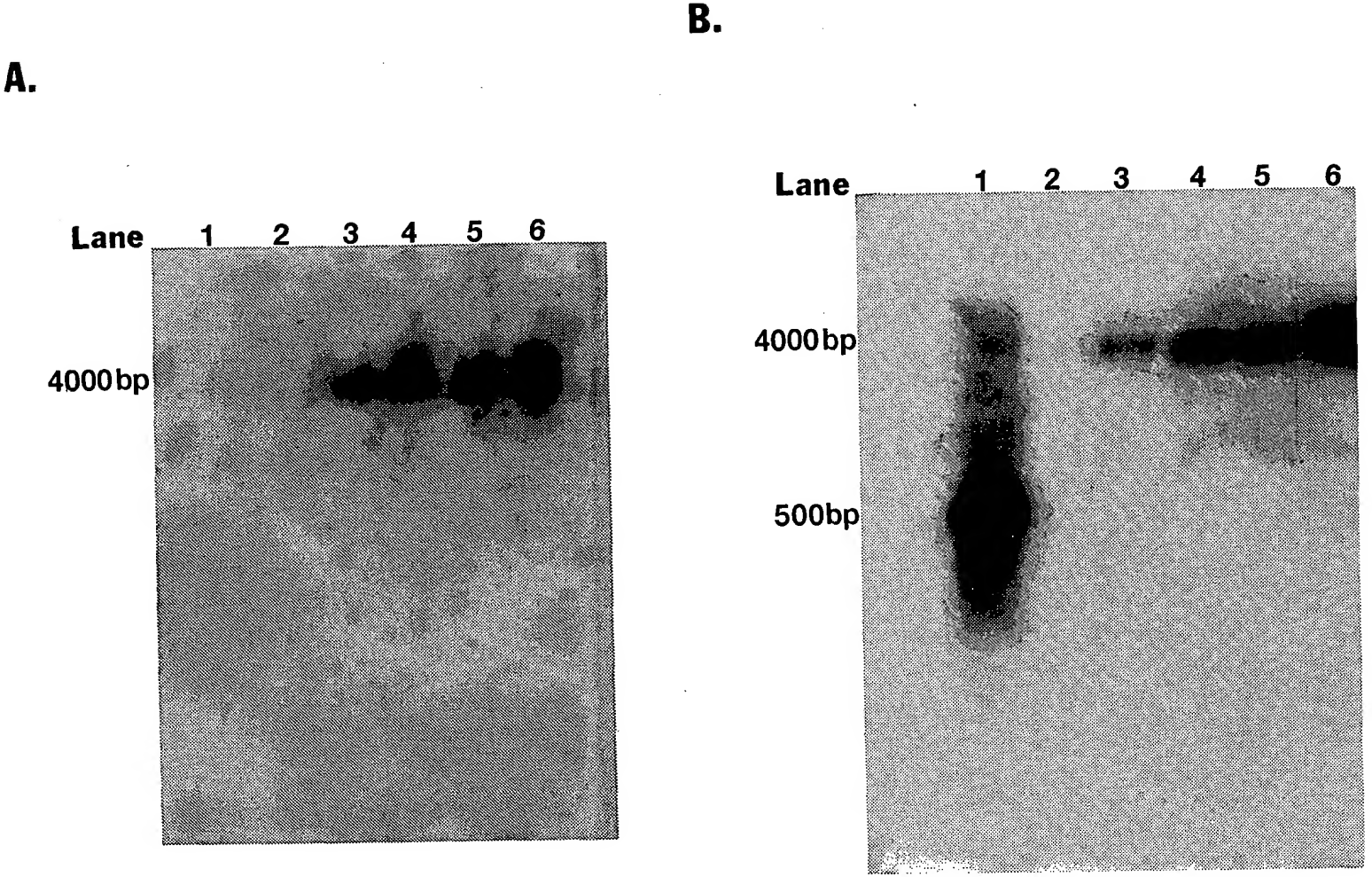



FIG 4.3

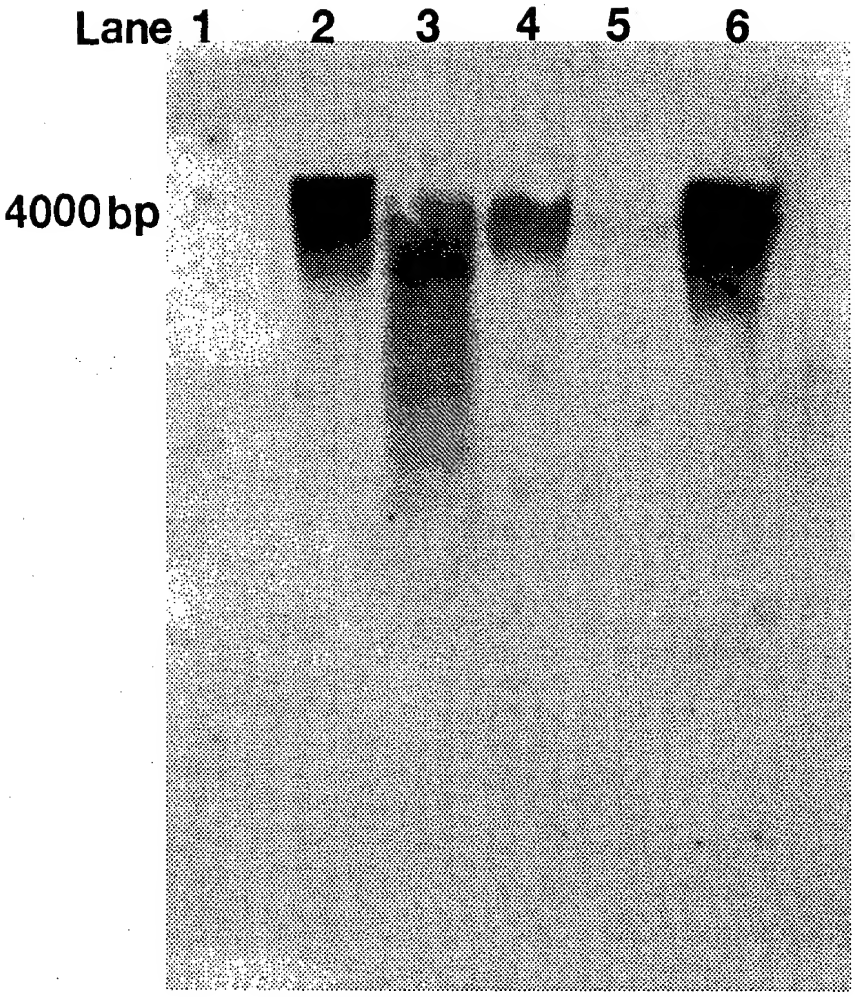



FIG 4.4

EXHIBIT 1049 - Page 74

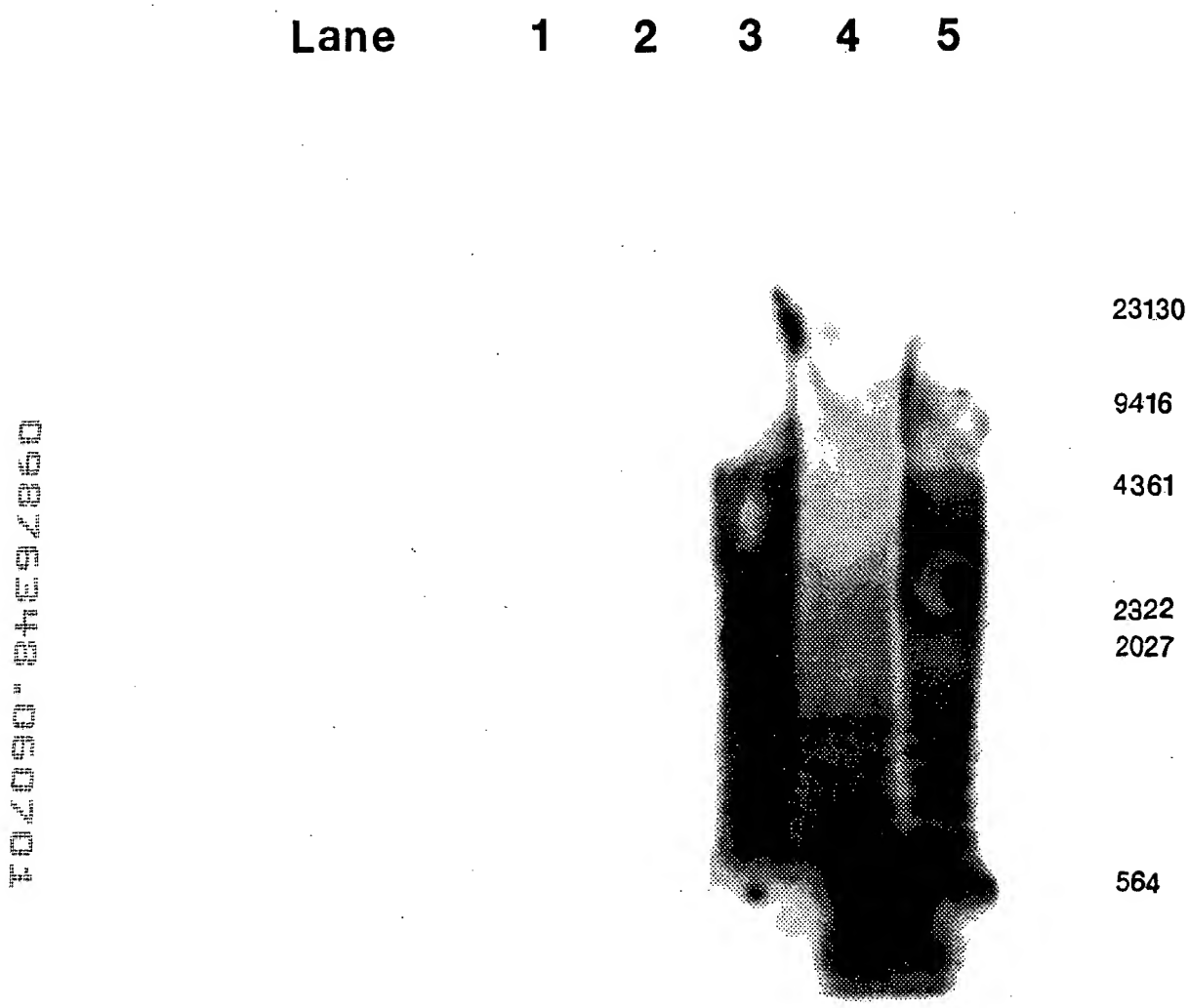



FIG 4.5

# Tm 13.17 cDNA

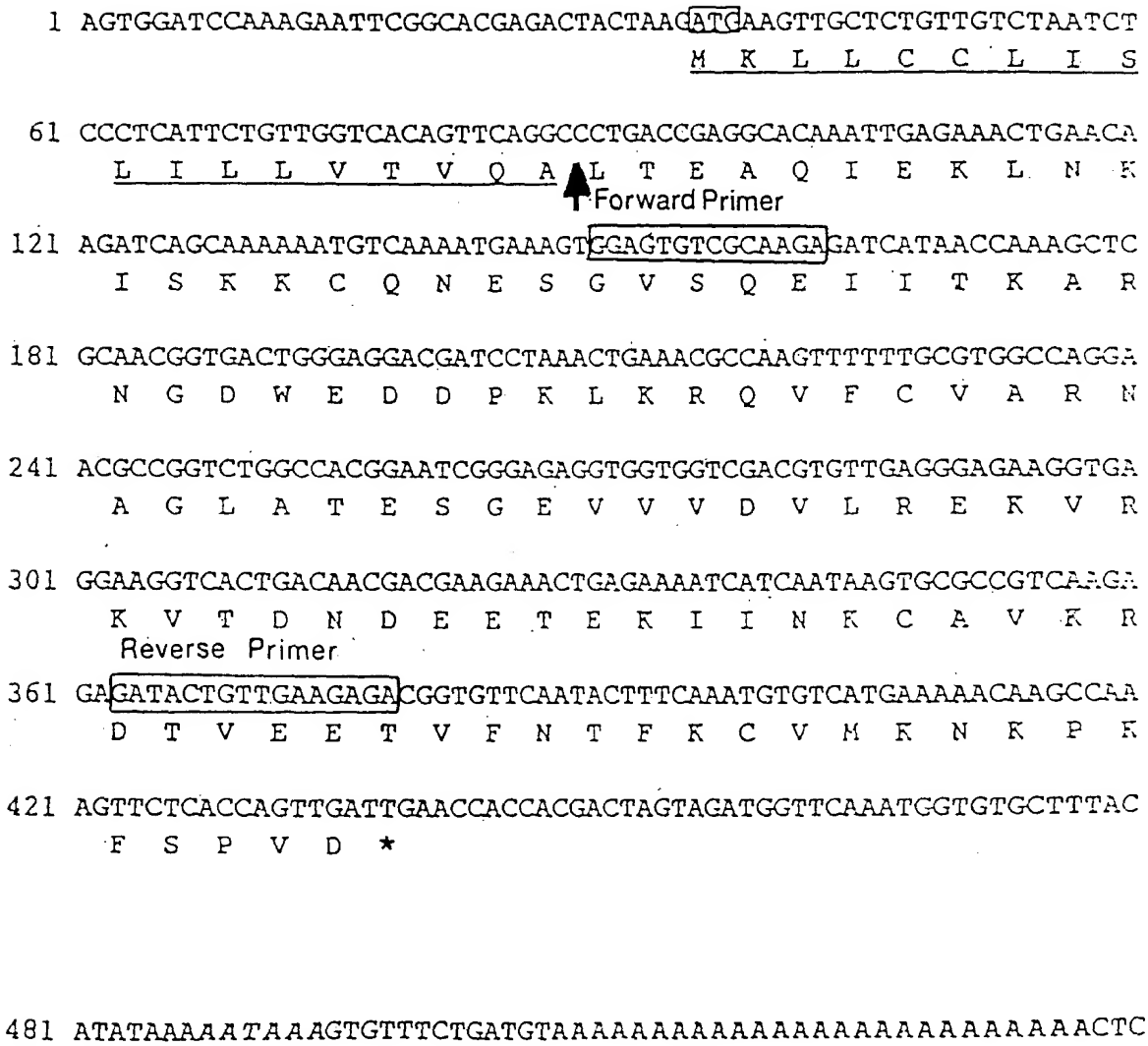



**FIG. 4.6 a**

**B.**

Forward Primer

```
2-2        L T D E Q I Q K R N K I S K E C Q Q V S G V S Q E T I D K V R T G V L V
Tm 13.17   L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A R N G D W E
B2         L T E E D L Q L L R Q T S A E C K T E S G A S E A V I K K A R K G D L E
AFP-3        E T P R E K L K Q H S D A C K A E S G V S E E S L N K V R N R E E V

2-2        D D P K M K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H
Tm 13.17   D D P K L K R Q V F C V A R N A G L A T E S G E V V V D V L R E K V R K
B2         D D P K L K M Q L L C I F K A L E I V A E S G E I E A D T F K E K L T R
AFP-3      D D P K L K E H A F C I L K R A G F I D A S G E F Q L D H I K T K F K E
```

Reverse Primer

```
2-2        V A S   D E E V D K I V Q K C V V K K A T P E E T A Y D T F K C I Y D S
Tm 13.17   V T D N D E E T E K I I N K C A V K R D T V E E T V F N T F K C V M K N
B2         V T N D D E E S E K I V E K C T V T E D T P E D T A F E V T K C V L K D
AFP-3      N S E H P E K V D D L V A K C A V K K D T P Q H S S A D F F K C V H D N

2-2        K P D F S P I D
Tm 13.17   K P K F S P V D
B2         K P N F F G D L F V
AFP-3      R S
```

**C.**

| Primer | percent % composition A | C | G | T | Melting Temperature ($^{o}$C) |
|---|---|---|---|---|---|
| Forward | 28.6 | 14.3 | 42.9 | 14.3 | 44.0 |
| Reverse | 25.0 | 31.3 | 6.3 | 37.5 | 44.0 |

FIG 4.6

Copyright © 1989 Genentech, Inc.

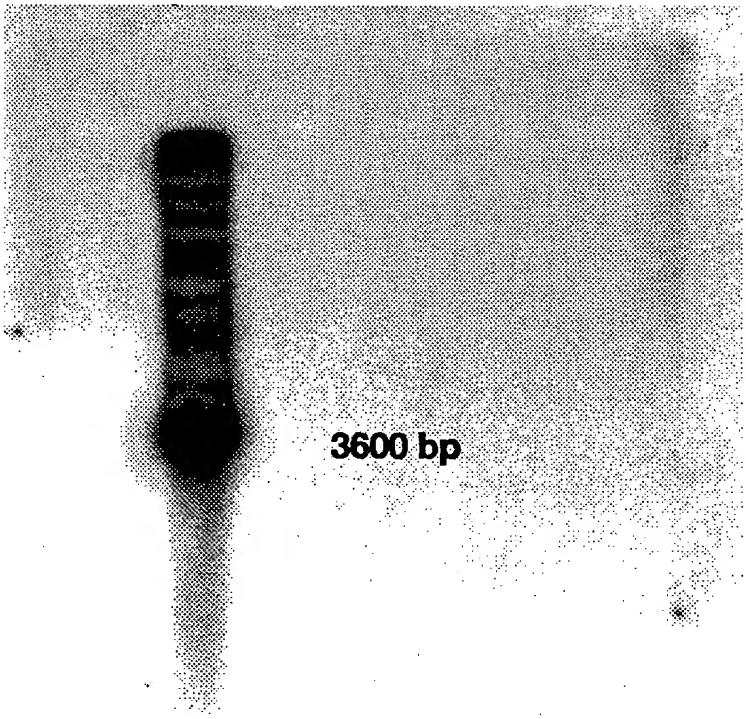



FIG 4.7

EP 0 956 868 A1

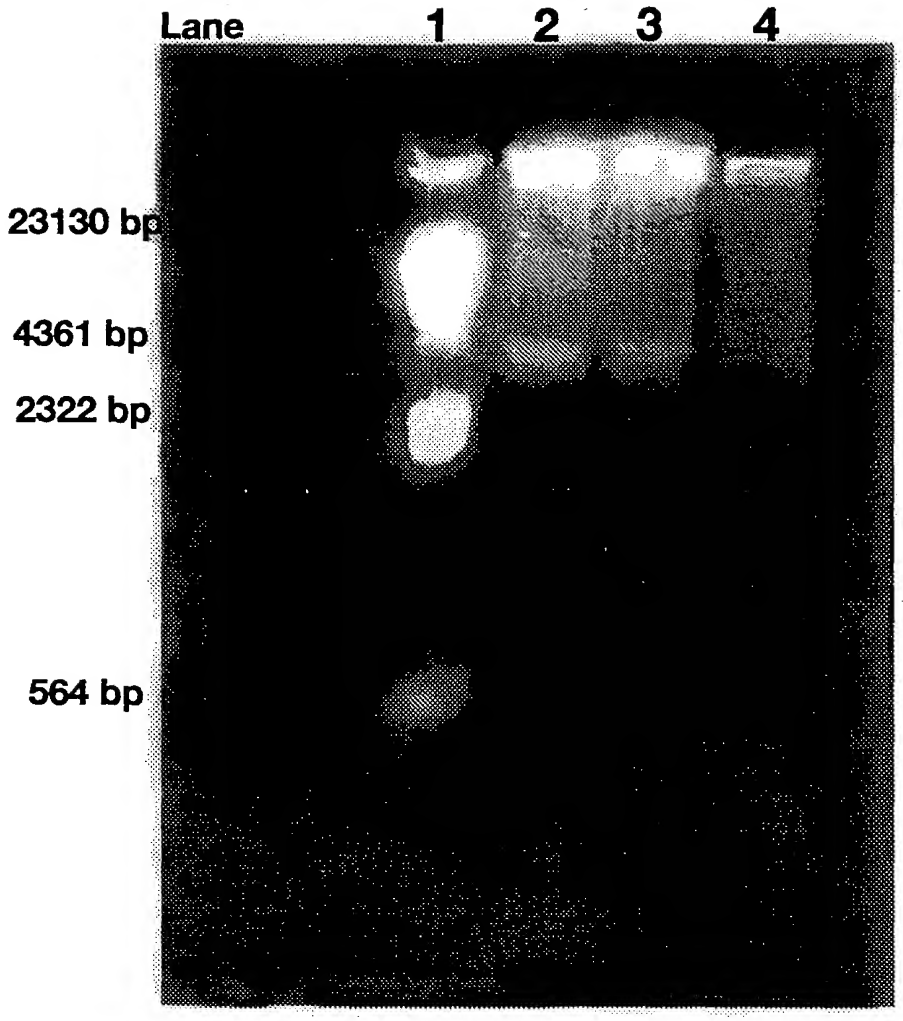



FIG 4.8

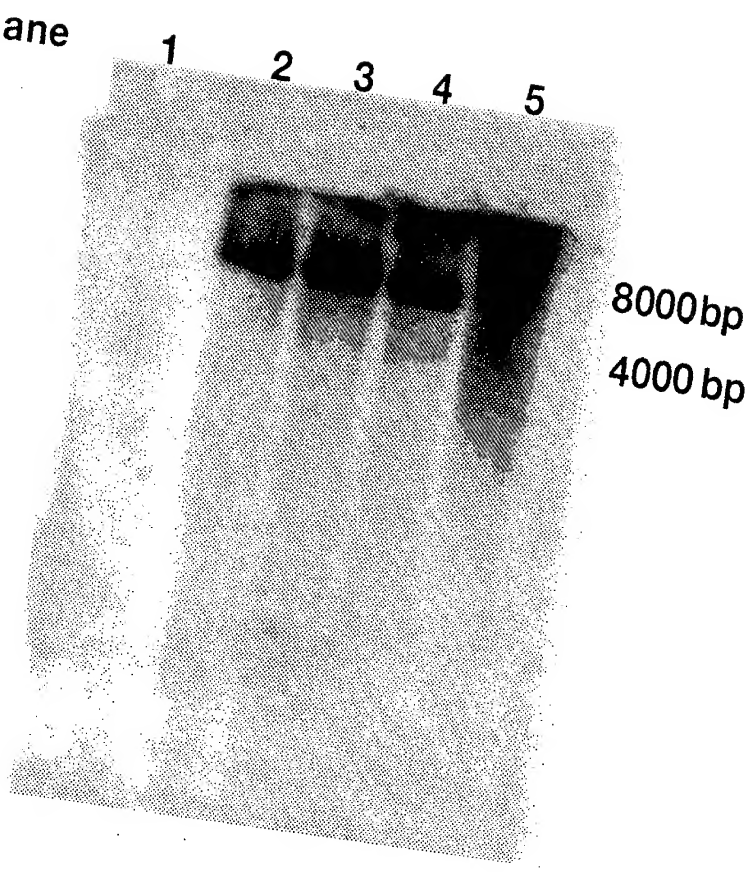



FIG 4.9

EP 0 507 639 A2

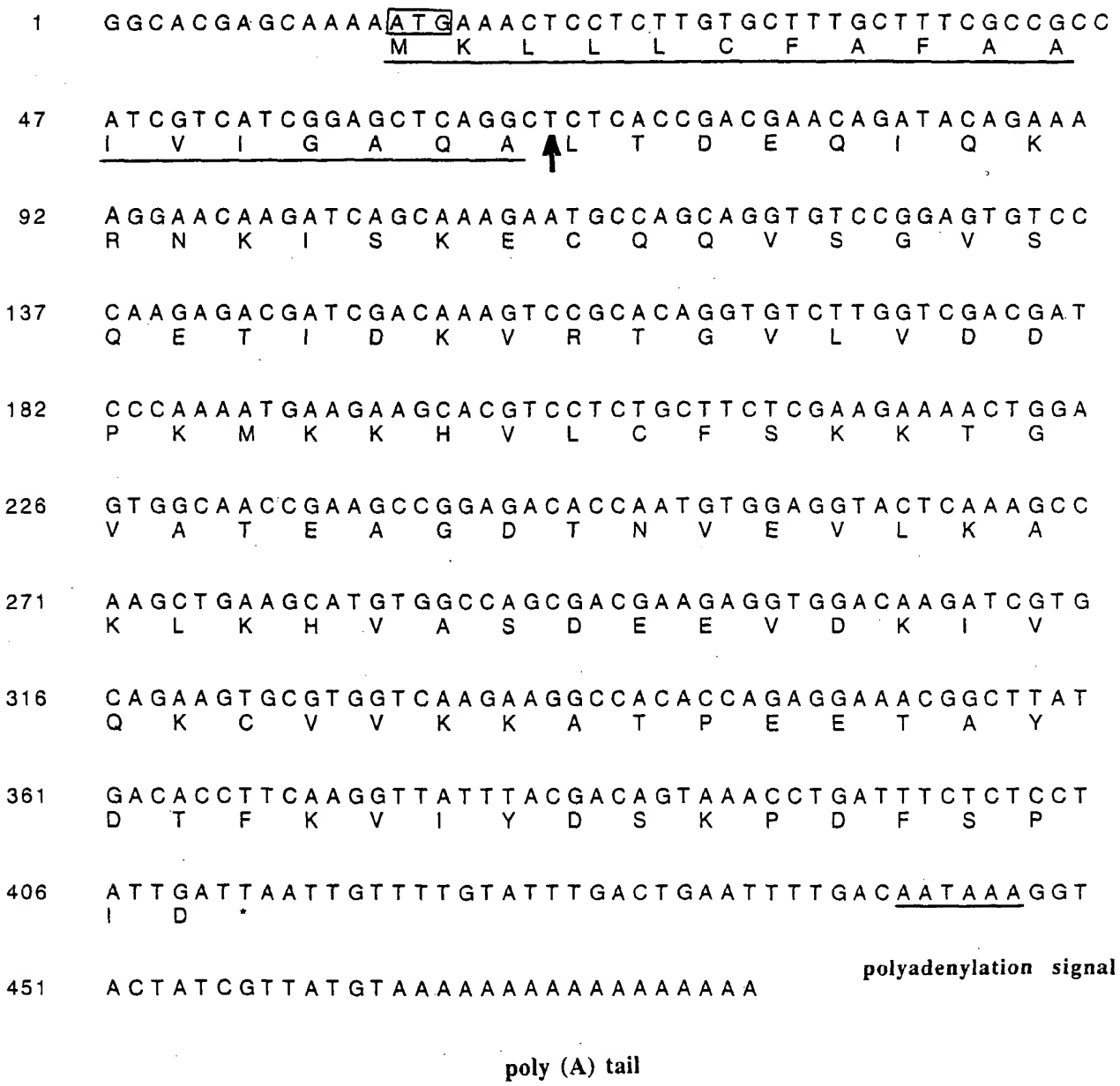



**FIG. 4.10 a**

| Analysis | Whole Protein |
|---|---|
| Molecular Weight | 12839.70 m.w. |
| Length | 115 |
| 1 microgram = | 77.883 pMoles |
| Molar Extinction coefficient | 2920±5% |
| 1 A(280) = | 4.40 mg/ml |
| Isoelectric Point | 7.14 |
| Charge at pH 7 | 0.16 |

Predicted Amino Acid

Composition of 3-4

Whole Protein Composition Analysis

| Amino Acid(s) | Number count | % by weight | % by frequency |
|---|---|---|---|
| Charged (RKHYCDE) | 47 | 46.41 | 40.87 |
| Acidic (DE) | 20 | 18.91 | 17.39 |
| Basic (KR) | 20 | 20.41 | 17.39 |
| Polar (NCQSTY) | 29 | 24.55 | 25.22 |
| Hydrophobic (AILFWV) | 35 | 28.04 | 30.43 |
| A Ala | 6 | 3.32 | 5.22 |
| C Cys | 3 | 2.41 | 2.61 |
| D Asp | 11 | 9.86 | 9.57 |
| E Glu | 9 | 9.05 | 7.83 |
| F Phe | 3 | 3.44 | 2.61 |
| G Gly | 4 | 1.78 | 3.48 |
| H His | 2 | 2.14 | 1.74 |
| I Ile | 6 | 5.29 | 5.22 |
| K Lys | 18 | 17.97 | 15.65 |
| L Leu | 5 | 4.41 | 4.35 |
| M Met | 1 | 1.02 | 0.87 |
| N Asn | 2 | 1.78 | 1.74 |
| P Pro | 4 | 3.02 | 3.48 |
| Q Gln | 6 | 5.99 | 5.22 |
| R Arg | 2 | 2.43 | 1.74 |
| S Ser | 7 | 4.75 | 6.09 |
| T Thr | 9 | 7.09 | 7.83 |
| V Val | 15 | 11.58 | 13.04 |
| W Trp | 0 | 0.00 | 0.00 |
| Y Tyr | 2 | 2.54 | 1.74 |
| B Asx | 0 | 0.00 | 0.00 |
| Z Glx | 0 | 0.00 | 0.00 |
| X Xxx | 0 | 0.00 | 0.00 |
| . Ter | 0 | 0.00 | 0.00 |

PCT/US98/05845 - 09/05/2000

**FIG. 4.10 b**

EP 0 369 683 A2

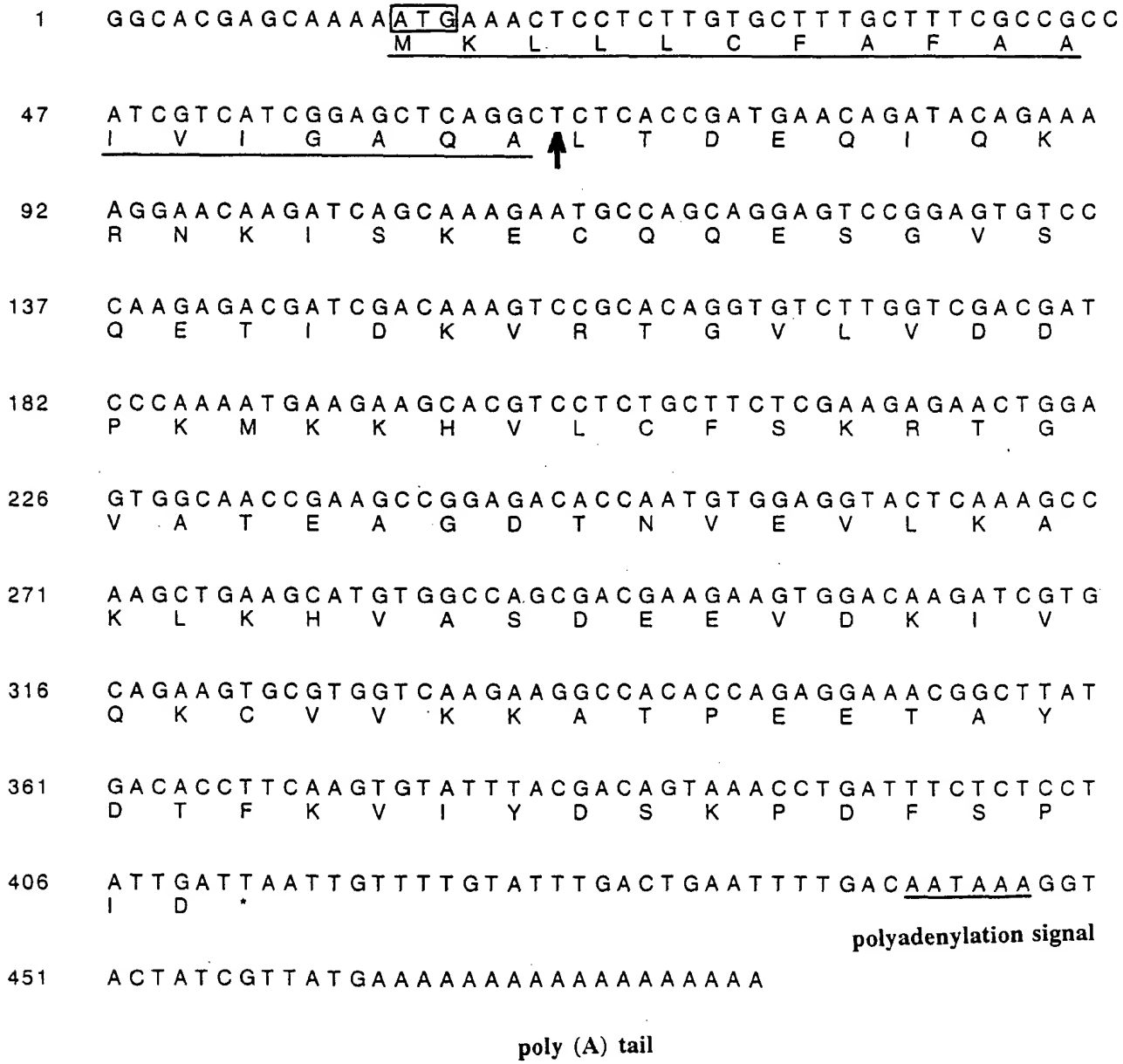



**FIG. 4.11 a**

Predicted Amino Acid

Composition of 3-9

| Analysis | Whole Protein |
|---|---|
| Molecular Weight | 12871.80 m.w. |
| Length | 115 |
| 1 microgram = | 77.689 pMoles |
| Molar Extinction coefficient | 3040±5% |
| 1 A(280) = | 4.23 mg/ml |
| Isoelectric Point | 7.11 |
| Charge at pH 7 | 0.13 |

Whole Protein Composition Analysis

| Amino Acid(s) | Number count | % by weight | % by frequency |
|---|---|---|---|
| Charged (RKHYCDE) | 48 | 47.31 | 41.74 |
| Acidic (DE) | 20 | 18.86 | 17.39 |
| Basic (KR) | 20 | 20.57 | 17.39 |
| Polar (NCQSTY) | 30 | 25.29 | 26.09 |
| Hydrophobic (AILFWV) | 34 | 27.20 | 29.57 |
| A Ala | 6 | 3.31 | 5.22 |
| C Cys | 4 | 3.21 | 3.48 |
| D Asp | 11 | 9.84 | 9.57 |
| E Glu | 9 | 9.03 | 7.83 |
| F Phe | 3 | 3.43 | 2.61 |
| G Gly | 4 | 1.77 | 3.48 |
| H His | 2 | 2.13 | 1.74 |
| I Ile | 6 | 5.28 | 5.22 |
| K Lys | 17 | 16.93 | 14.78 |
| L Leu | 5 | 4.40 | 4.35 |
| M Met | 1 | 1.02 | 0.87 |
| N Asn | 2 | 1.77 | 1.74 |
| P Pro | 4 | 3.02 | 3.48 |
| Q Gln | 6 | 5.97 | 5.22 |
| R Arg | 3 | 3.64 | 2.61 |
| S Ser | 7 | 4.74 | 6.09 |
| T Thr | 9 | 7.07 | 7.83 |
| V Val | 14 | 10.78 | 12.17 |
| W Trp | 0 | 0.00 | 0.00 |
| Y Tyr | 2 | 2.54 | 1.74 |
| B Asx | 0 | 0.00 | 0.00 |
| Z Glx | 0 | 0.00 | 0.00 |
| X Xxx | 0 | 0.00 | 0.00 |
| . Ter | 0 | 0.00 | 0.00 |

P02958-B6N9G9

FIG. 4.11 b

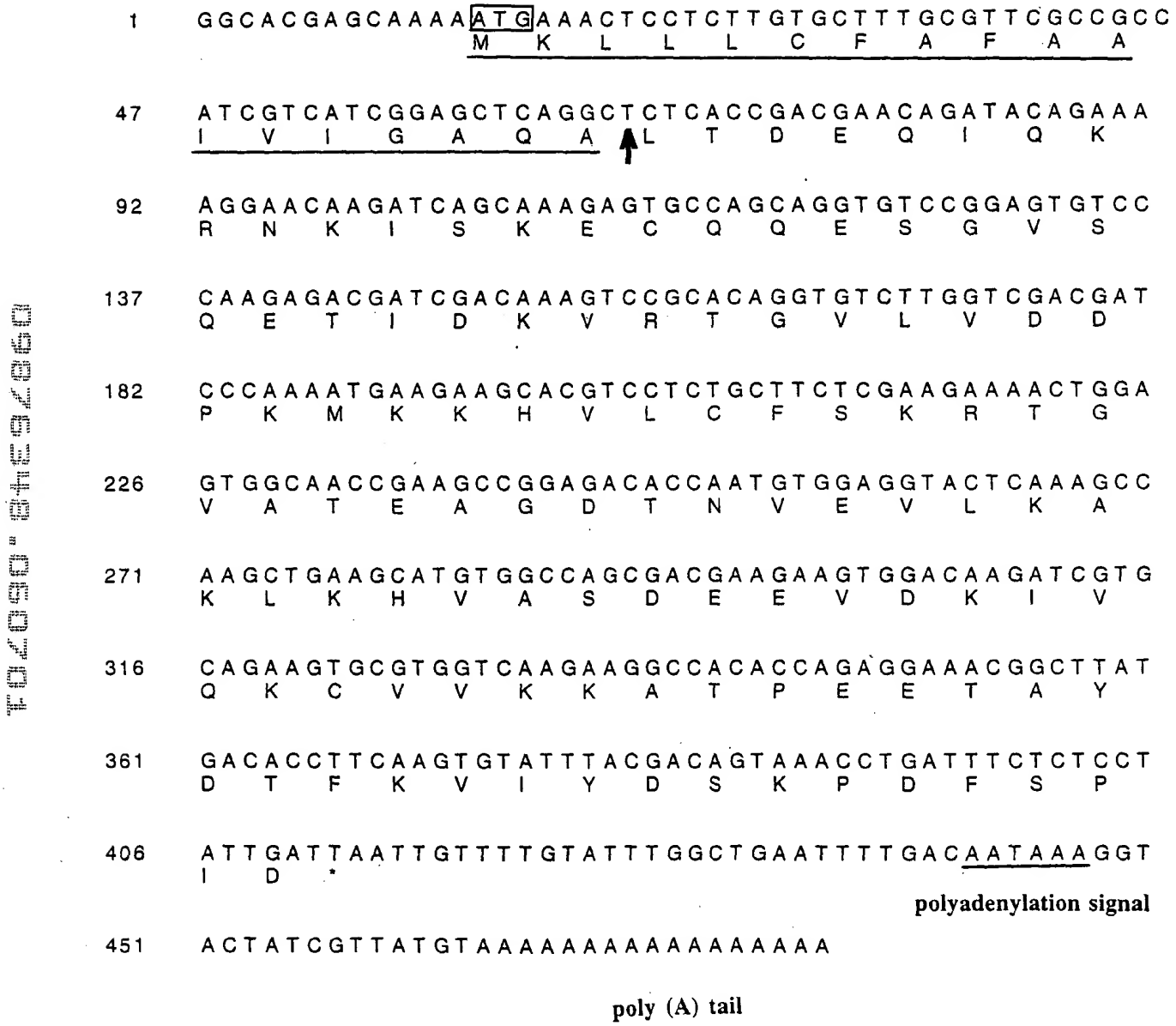

1 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC
M K L L L C F A F A A
47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
I V I G A Q A L T D E Q I Q K
92 AGGAACAAGATCAGCAAAGAGTGCCAGCAGGTGTCCGGAGTGTCC
R N K I S K E C Q Q E S G V S
137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
Q E T I D K V R T G V L V D D
182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
P K M K K H V L C F S K R T G
226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
V A T E A G D T N V E V L K A
271 AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
K L K H V A S D E E V D K I V
316 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
Q K C V V K K A T P E E T A Y
361 GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
D T F K V I Y D S K P D F S P
406 ATTGATTAATTGTTTTGTATTTGGCTGAATTTTGACAATAAAGGT
I D *
polyadenylation signal
451 ACTATCGTTATGTAAAAAAAAAAAAAAAAA
poly (A) tail

FIG. 4.12 a

Predicted Amino Acid

Composition of 7–5

| Analysis | Whole Protein |
|---|---|
| Molecular Weight | 12843.80 m.w. |
| Length | 115 |
| 1 microgram = | 77.859 pMoles |
| Molar Extinction coefficient | 3040±5% |
| 1 A(280) = | 4.22 mg/ml |
| Isoelectric Point | 7.11 |
| Charge at pH 7 | 0.13 |

Whole Protein Composition Analysis

| Amino Acid(s) | Number count | % by weight | % by frequency |
|---|---|---|---|
| Charged (RKHYCDE) | 48 | 47.19 | 41.74 |
| Acidic (DE) | 20 | 18.90 | 17.39 |
| Basic (KR) | 20 | 20.40 | 17.39 |
| Polar (NCQSTY) | 30 | 25.35 | 26.09 |
| Hydrophobic (AILFWV) | 34 | 27.26 | 29.57 |
| A Ala | 6 | 3.32 | 5.22 |
| C Cys | 4 | 3.21 | 3.48 |
| D Asp | 11 | 9.86 | 9.57 |
| E Glu | 9 | 9.05 | 7.83 |
| F Phe | 3 | 3.44 | 2.61 |
| G Gly | 4 | 1.78 | 3.48 |
| H His | 2 | 2.14 | 1.74 |
| I Ile | 6 | 5.29 | 5.22 |
| K Lys | 18 | 17.97 | 15.65 |
| L Leu | 5 | 4.41 | 4.35 |
| M Met | 1 | 1.02 | 0.87 |
| N Asn | 2 | 1.78 | 1.74 |
| P Pro | 4 | 3.02 | 3.48 |
| Q Gln | 6 | 5.98 | 5.22 |
| R Arg | 2 | 2.43 | 1.74 |
| S Ser | 7 | 4.75 | 6.09 |
| T Thr | 9 | 7.08 | 7.83 |
| V Val | 14 | 10.80 | 12.17 |
| W Trp | 0 | 0.00 | 0.00 |
| Y Tyr | 2 | 2.54 | 1.74 |
| B Asx | 0 | 0.00 | 0.00 |
| Z Glx | 0 | 0.00 | 0.00 |
| X Xxx | 0 | 0.00 | 0.00 |
| . Ter | 0 | 0.00 | 0.00 |

FIG. 4.12 b

EP 0 968 274 B1

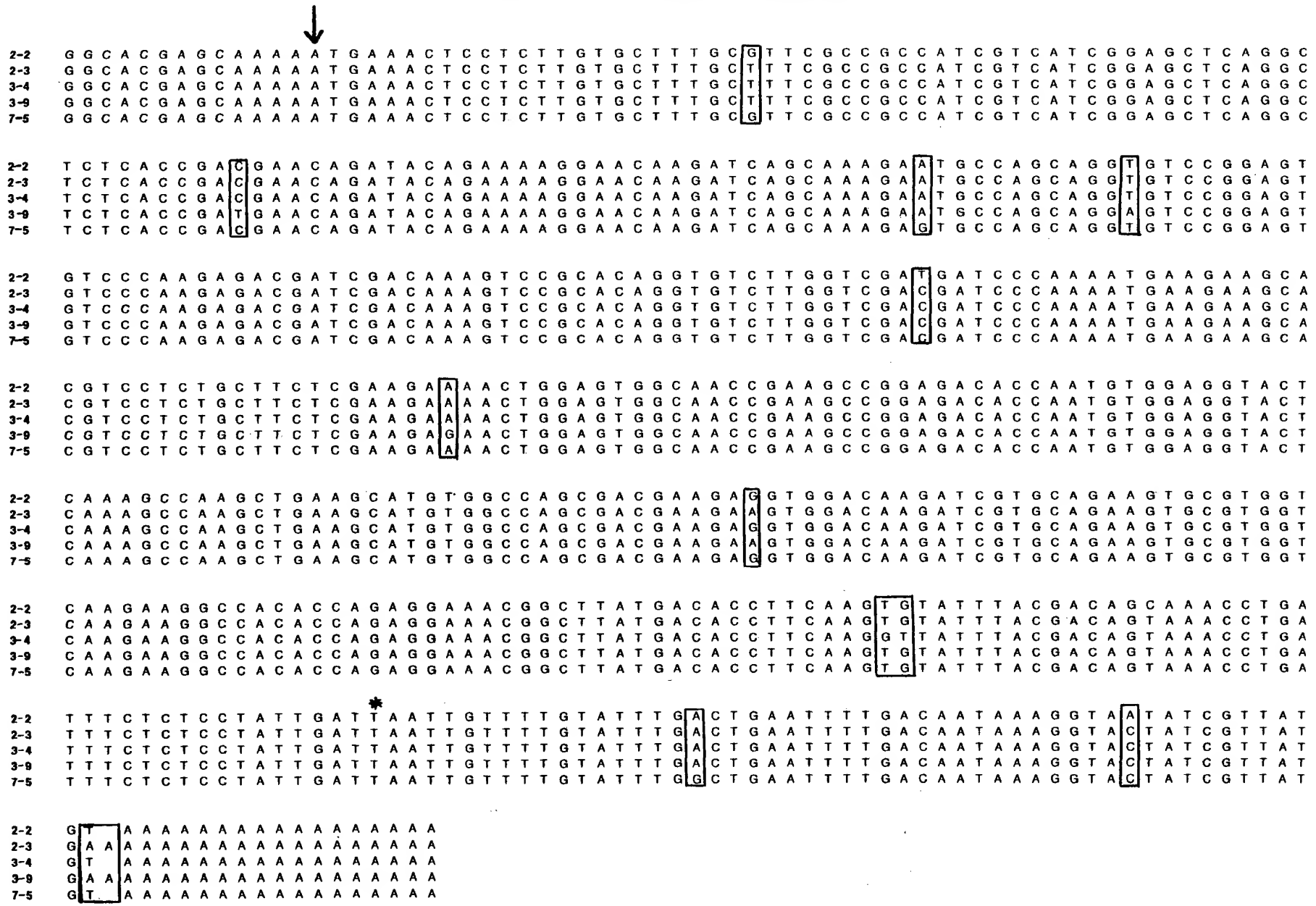

2-2 GGCACGAGCAAAAAATGAAAACTCCTCTTGTGCTTTGCGTTCGCCGCCATCGTCATCGGAGCTCAGGC
2-3 GGCACGAGCAAAAAATGAAAACTCCTCTTGTGCTTTGCTTTCGCCGCCATCGTCATCGGAGCTCAGGC
3-4 GGCACGAGCAAAAAATGAAAACTCCTCTTGTGCTTTGCTTTCGCCGCCATCGTCATCGGAGCTCAGGC
3-9 GGCACGAGCAAAAAATGAAAACTCCTCTTGTGCTTTGCTTTCGCCGCCATCGTCATCGGAGCTCAGGC
7-5 GGCACGAGCAAAAAATGAAAACTCCTCTTGTGCTTTGCGTTCGCCGCCATCGTCATCGGAGCTCAGGC
2-2 TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGT
2-3 TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGT
3-4 TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGT
3-9 TCTCACCGATGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAATGCCAGCAGGAGTCCGGAGT
7-5 TCTCACCGACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGAGTGCCAGCAGGTGTCCGGAGT
2-2 GTCCCAAGAGACGATCGACAAAAGTCCGCACAGGTGTCTTGGTCGATGATCCCAAAAATGAAGAAGCA
2-3 GTCCCAAGAGACGATCGACAAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAAATGAAGAAGCA
3-4 GTCCCAAGAGACGATCGACAAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAAATGAAGAAGCA
3-9 GTCCCAAGAGACGATCGACAAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAAATGAAGAAGCA
7-5 GTCCCAAGAGACGATCGACAAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCAAAAATGAAGAAGCA
2-2 CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
2-3 CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
3-4 CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
3-9 CGTCCTCTGCTTCTCGAAGAGAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
7-5 CGTCCTCTGCTTCTCGAAGAAAACTGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACT
2-2 CAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGT
2-3 CAAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTGCAGAAGTGCGTGGT
3-4 CAAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGT
3-9 CAAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTGCAGAAGTGCGTGGT
7-5 CAAAAGCCAAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGT
2-2 CAAGAAGGCCACACCCAGAGGGAAAACGGCTTATGACACCTTCAAAGTGTATTTACGACAGCAAACCTGA
2-3 CAAGAAGGCCACACCCAGAGGGAAAACGGCTTATGACACCTTCAAAGTGTATTTACGACAGTAAACCTGA
3-4 CAAGAAGGCCACACCCAGAGGGAAAACGGCTTATGACACCTTCAAAGGTTATTTACGACAGTAAACCTGA
3-9 CAAGAAGGCCACACCCAGAGGAAAACGGCTTATGACACCTTCAAAGTGTATTTACGACAGTAAACCTGA
7-5 CAAGAAGGCCACACCCAGAGGAAAACGGCTTATGACACCTTCAAAGTGTATTTACGACAGTAAACCTGA
2-2 TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTAATATCGTTAT
2-3 TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTACTATCGTTAT
3-4 TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTACTATCGTTAT
3-9 TTTCTCTCCTATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGTACTATCGTTAT
7-5 TTTCTCTCCTATTGATTAATTGTTTTGTATTTGGCTGAATTTTGACAATAAAGGTACTATCGTTAT
2-2 GTAAAAAAAAAAAAAAAAAAAA
2-3 GAAAAAAAAAAAAAAAAAAAAA
3-4 GT AAAAAAAAAAAAAAAAAAAA
3-9 GAAAAAAAAAAAAAAAAAAAAA
7-5 GT AAAAAAAAAAAAAAAAAAAA

FIG. 4.13

TD2090 "8hE92860

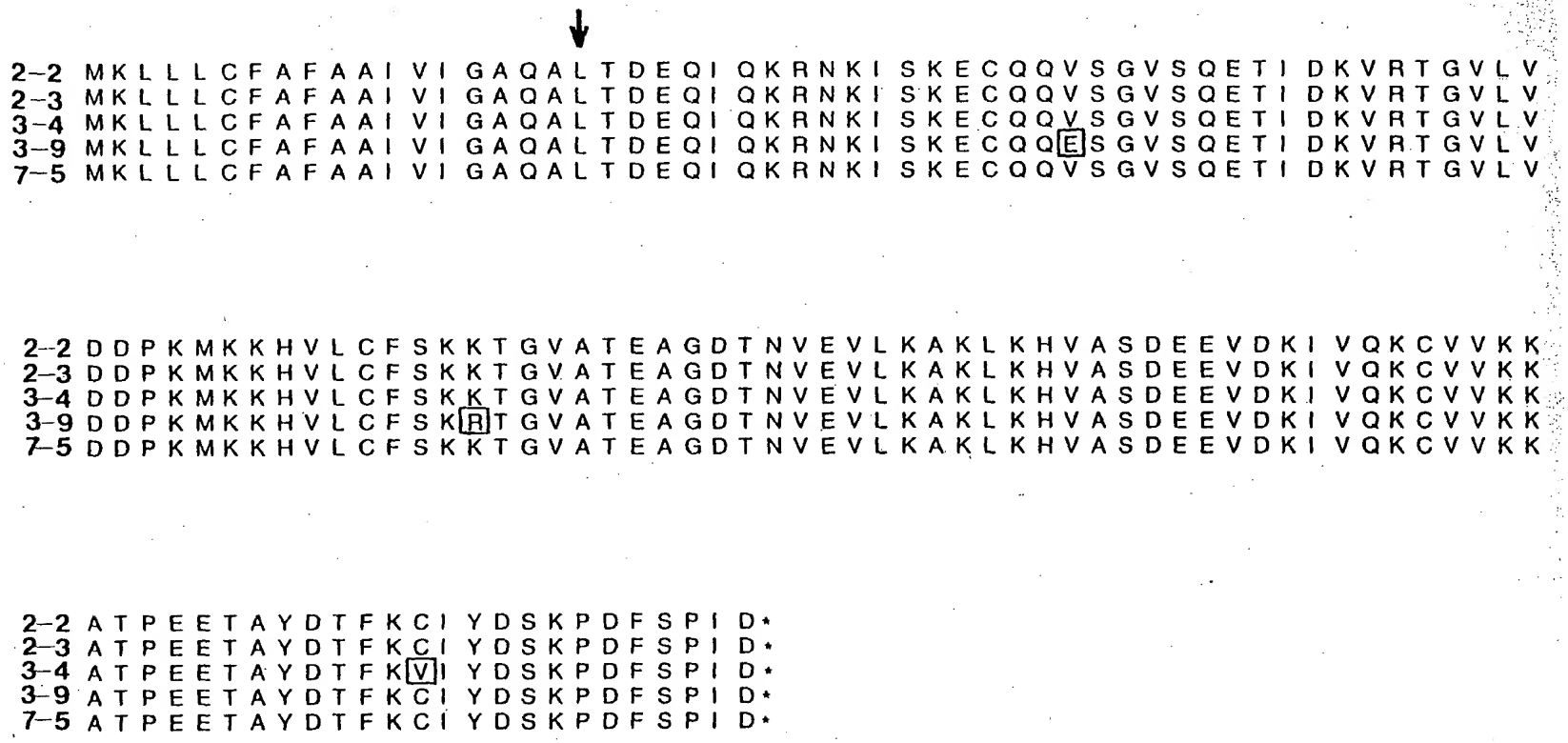



**FIG. 4.14**

| | (kDa) | (% mole) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **MW** | **AA** | **Cys** | **Pro** | **Phe** | **Ile** | **Val** | **Met** | **Leu** | % most hydrophobic | **Gly** | **Ala** | **Tyr** | **His** | **Trp** | **Asx** | **Glx** | **Arg** | **Lys** | **Ser** | **Thr** | % most hydrophilic |
| **Tm 12.86** | 12.86 | 117 | 3.2 | 3.0 | 3.4 | 4.4 | 8.5 | 2.0 | 4.4 | 28.9 | 3.1 | 3.9 | 3.8 | 3.2 | ND | 10.7 | 15.0 | 3.6 | 14.9 | 6.8 | 6.3 | 57.3 |
| **Tm 13.17** | 13.17 | 116 | 3.13 | 2.21 | 4.47 | 5.16 | 10.5 | 1.0 | 4.3 | 29.11 | 1.73 | 3.24 | 0 | 0 | 1.41 | 0 | 0 | 7.12 | 15.6 | 3.31 | 6.14 | 32.14 |
| **2-2** | 12.84 | 115 | 3.21 | 3.02 | 3.44 | 5.29 | 10.8 | 1.02 | 4.41 | 27.26 | 1.78 | 3.32 | 2.54 | 2.14 | 0 | 0 | 0 | 2.43 | 18.0 | 4.75 | 7.08 | 32.23 |
| **2-3** | 12.84 | 115 | 3.21 | 3.02 | 3.44 | 5.29 | 10.8 | 1.02 | 4.41 | 27.26 | 1.78 | 3.32 | 2.54 | 2.14 | 0 | 0 | 0 | 2.43 | 18.0 | 4.75 | 7.08 | 32.23 |
| **3-4** | 12.84 | 115 | 2.41 | 3.02 | 3.44 | 5.29 | 11.6 | 1.02 | 4.41 | 28.04 | 1.78 | 3.32 | 2.54 | 2.14 | 0 | 0 | 0 | 2.43 | 18.0 | 4.75 | 7.09 | 32.24 |
| **3-9** | 12.87 | 115 | 3.21 | 3.02 | 3.43 | 5.28 | 10.8 | 1.02 | 4.40 | 27.20 | 1.77 | 3.31 | 2.54 | 2.13 | 0 | 0 | 0 | 3.64 | 16.9 | 4.74 | 7.07 | 32.38 |
| **7-5** | 12.84 | 115 | 3.21 | 3.02 | 3.44 | 5.29 | 10.8 | 1.02 | 4.41 | 27.26 | 1.78 | 3.32 | 2.54 | 2.14 | 0 | 0 | 0 | 2.43 | 18.0 | 4.75 | 7.08 | 32.23 |

**FIG. 4.15**

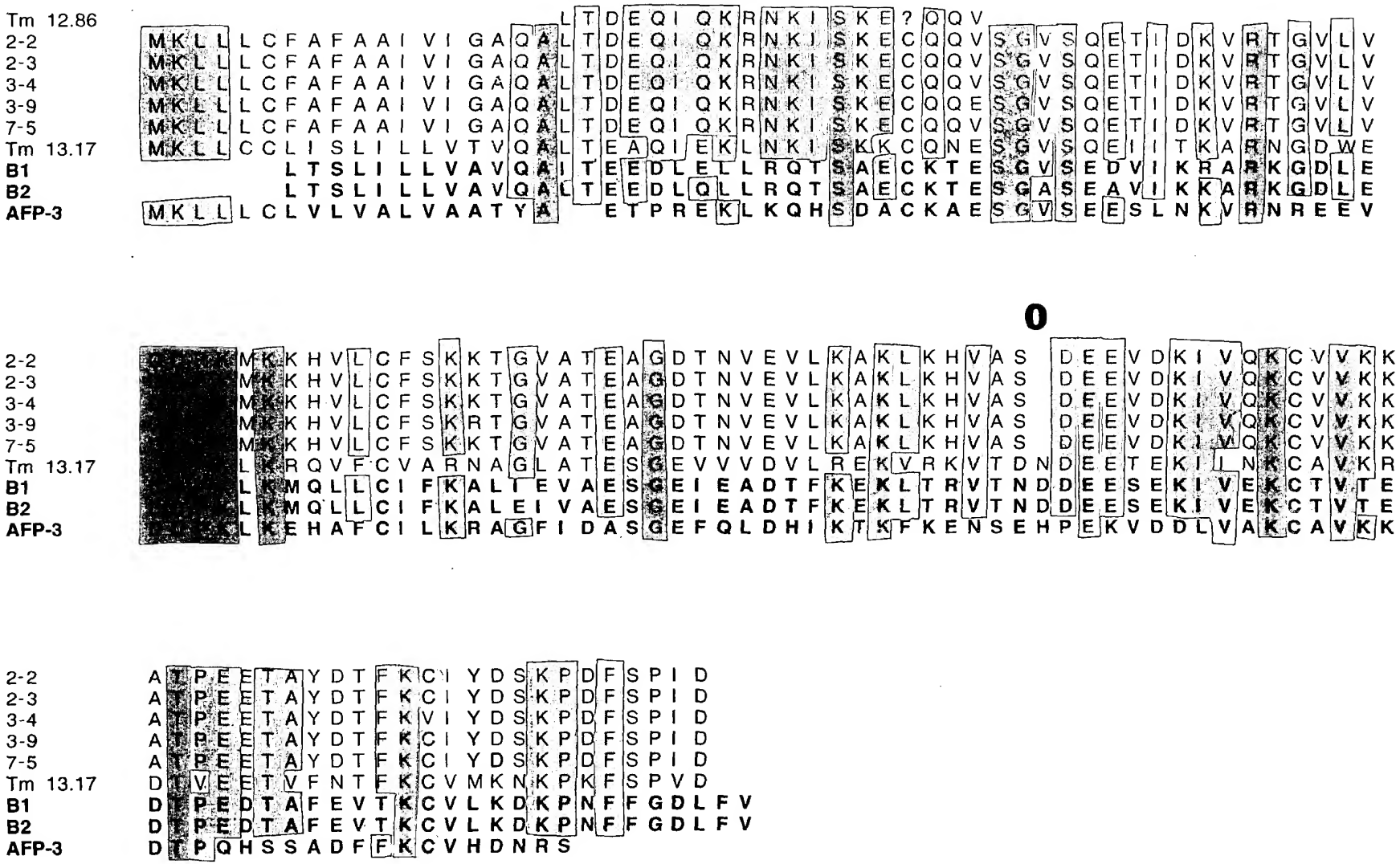



FIG. 4.16

T02090 "8hE92860

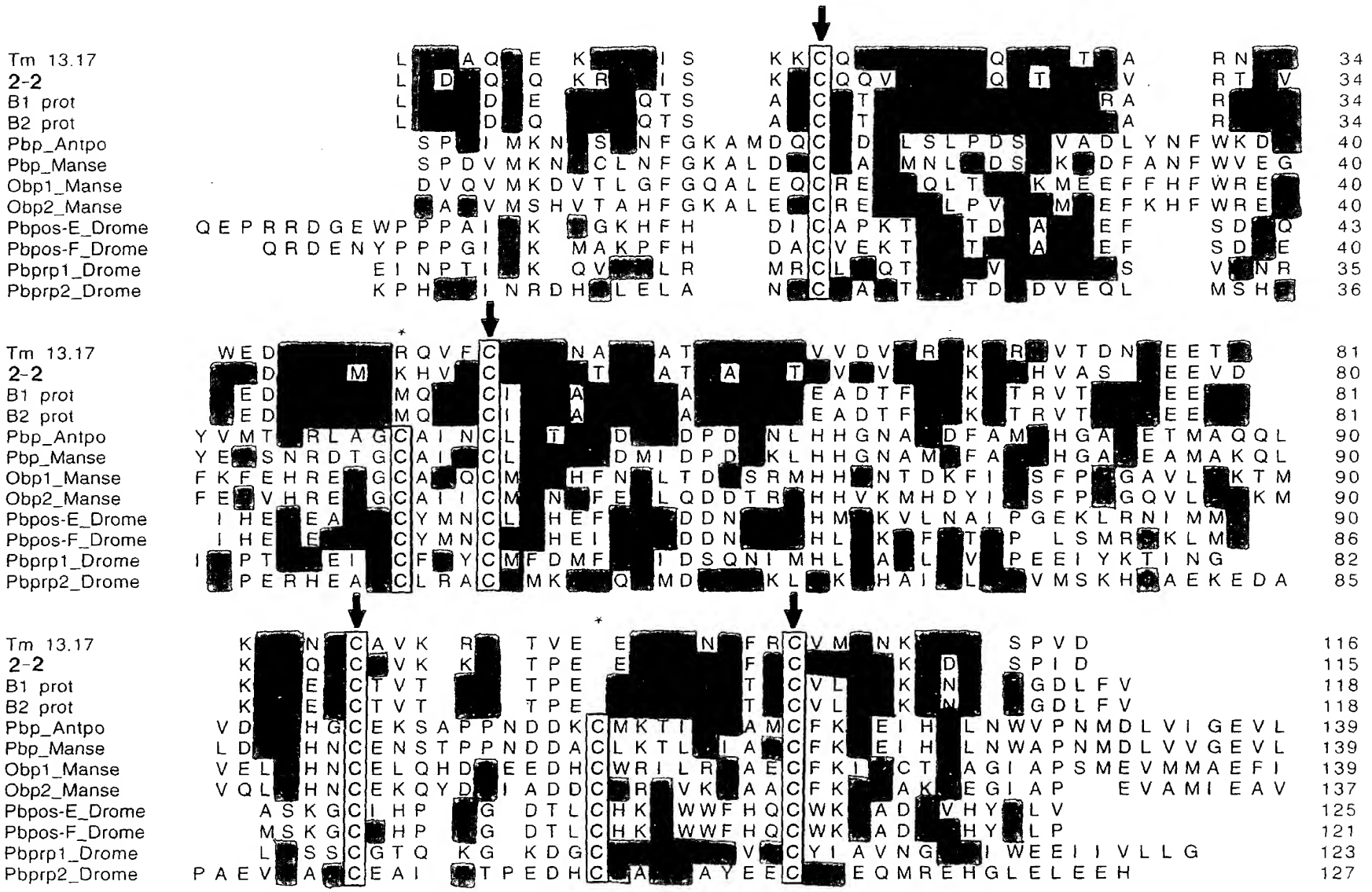



FIG. 4.17

T07090 08E97860

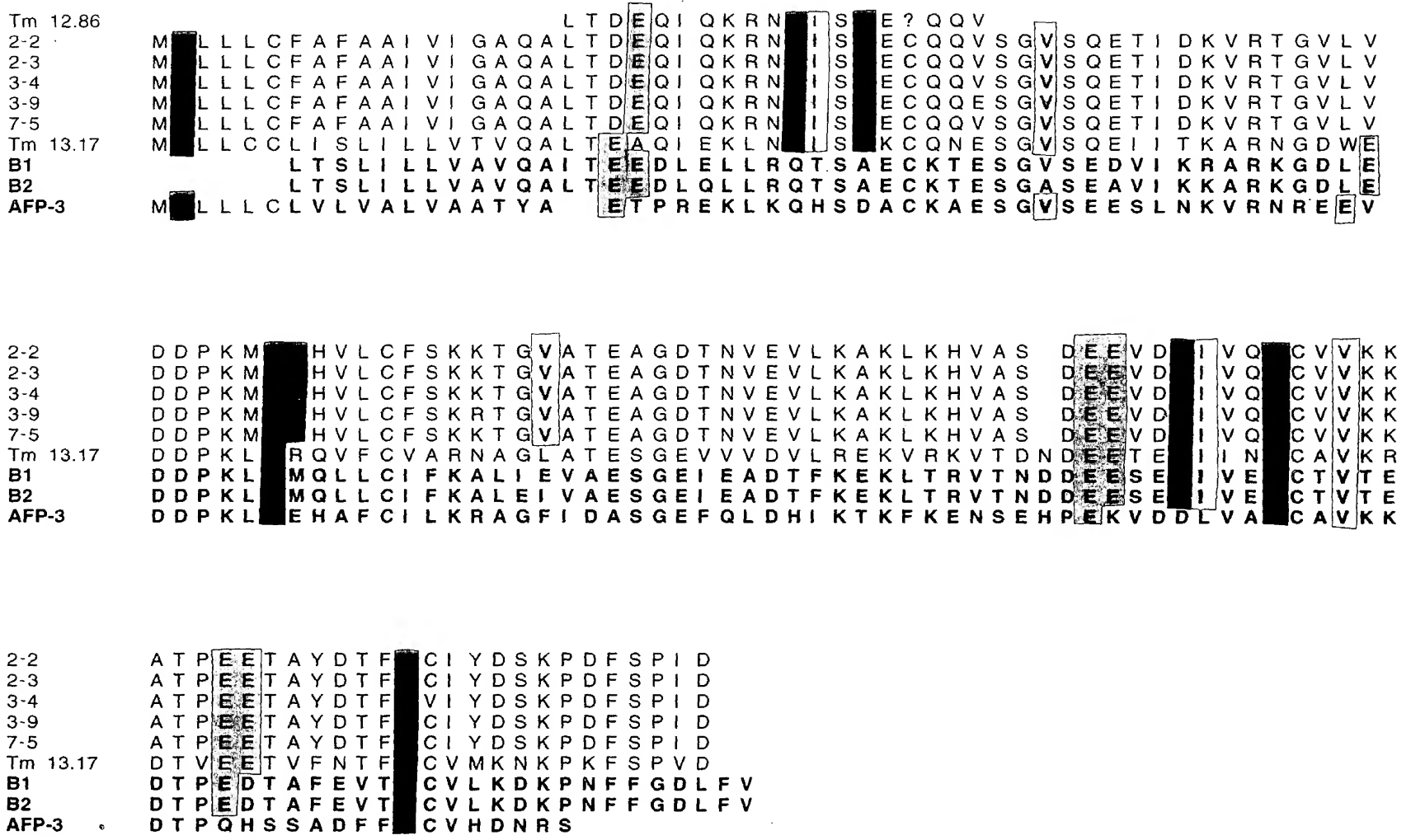



FIG. 4.18

# NUCLEOTIDE SEQUENCES

PERCENT SIMILARITY

| | 1 (2-2) | 2 (2-3) | 3 (3-4) | 4 (3-9) | 5 (7-5) | 6 (Tm13.17) | 7 (B1) | 8 (AFP-3) | 9 (YL-1) | 10 (DAFP-1A) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 99.1 | 97.3 | 98.4 | 98.2 | 50.4 | 42 | 42.4 | 20.7 | 20.9 | 1 | 2-2 |
| 2 | 0.9 | | 99 | 99 | 99 | 51.5 | 43.2 | 42.7 | 20.2 | 21.3 | 2 | 2-3 |
| 3 | 1.6 | 60 | | 98 | 98 | 50.3 | 42 | 42.1 | 20 | 20 | 3 | 3-4 |
| 4 | 1.6 | 20 | 80 | | 98.4 | 51.3 | 43.7 | 43.6 | 20.4 | 22 | 4 | 3-9 |
| 5 | 1.8 | 25 | 75 | 45 | | 50.4 | 42.5 | 42.9 | 22.2 | 23.1 | 5 | 7-5 |
| 6 | 36.9 | 36.4 | 37.2 | 36.4 | 37.1 | | 57.2 | 37.4 | 23.3 | 21 | 6 | Tm 13.17 |
| 7 | 39.9 | 41.2 | 41.7 | 40.7 | 41.2 | 30.2 | | 39.3 | 21.9 | 22.1 | 7 | B1 |
| 8 | 41.8 | 42.4 | 43.1 | 42.3 | 42.3 | 45.3 | 49.4 | | 21.8 | 23.1 | 8 | AFP-3 |
| 9 | 48.7 | 58.6 | 52 | 51.2 | 51.9 | 61.7 | 62.2 | 58.2 | | 45.6 | 9 | YL-1 |
| 10 | 51.4 | 62.7 | 46.4 | 46.5 | 46.5 | 60 | 58.1 | 61.8 | 35.5 | | 10 | DAFP-1A |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | |

PERCENT DIVERGENCE

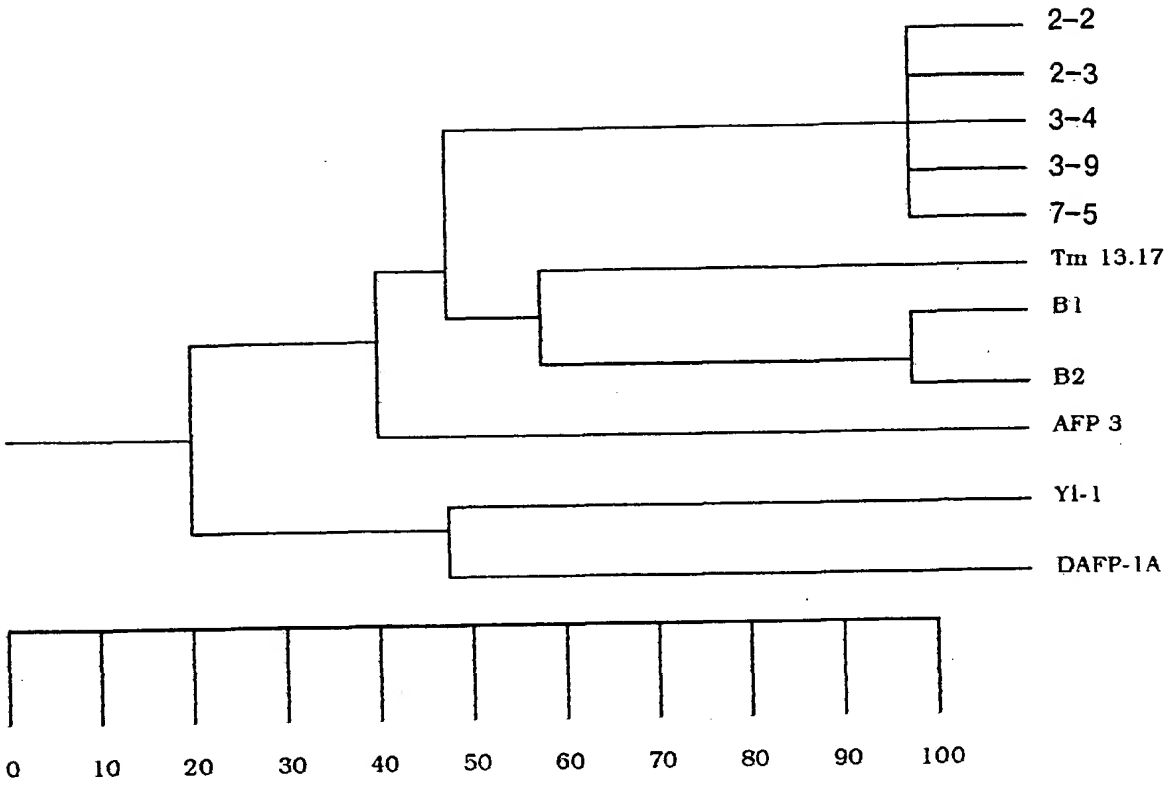
# AMINO ACID SEQUENCES

PERCENT SIMILARITY

| | 1 (2-2) | 2 (2-3) | 3 (3-4) | 4 (3-9) | 5 (7-5) | 6 (Tm13.17) | 7 (B1) | 8 (AFP-3) | 9 (YL-1) | 10 (DAFP-1A) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 100 | 99.1 | 99.1 | 100 | 51.3 | 37.4 | 35.2 | 11.6 | 12 | 1 | 2-2 |
| 2 | 0 | | 99.1 | 99.1 | 100 | 51.3 | 37.4 | 35.2 | 11.6 | 12 | 2 | 2-3 |
| 3 | 0.9 | 0.9 | | 98.3 | 99.1 | 50.4 | 36.5 | 34.3 | 11.6 | 12 | 3 | 3-4 |
| 4 | 0.9 | 0.9 | 1.7 | | 99.1 | 51.3 | 37.4 | 36.1 | 10.7 | 12 | 4 | 3-9 |
| 5 | 0 | 0 | 0.9 | 0.09 | | 51.3 | 37.4 | 35.2 | 11.6 | 12 | 5 | 7-5 |
| 6 | 46.1 | 46.1 | 47 | 46.1 | 46.1 | | 47.4 | 39.8 | 13.4 | 13.9 | 6 | Tm 13.17 |
| 7 | 59.1 | 59.1 | 60 | 59.1 | 59.1 | 51.7 | | 37 | 11.6 | 11.1 | 7 | B1 |
| 8 | 61.7 | 61.7 | 62.6 | 60.7 | 61.7 | 60.2 | 63 | | 10.2 | 8.3 | 8 | AFP-3 |
| 9 | 86.7 | 86.7 | 85.7 | 85.7 | 85.7 | 86.8 | 84.2 | 87.5 | | 55.6 | 9 | YL-1 |
| 10 | 88.3 | 88.3 | 86.4 | 84.5 | 85.4 | 89.1 | 82.7 | 90.5 | 40.2 | | 10 | DAFP-1A |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | |

PERCENT DIVERGENCE

FIG 4.19




% Nucleic Acid Identity

FIG 4.20

EFS Web - 04/30/2010

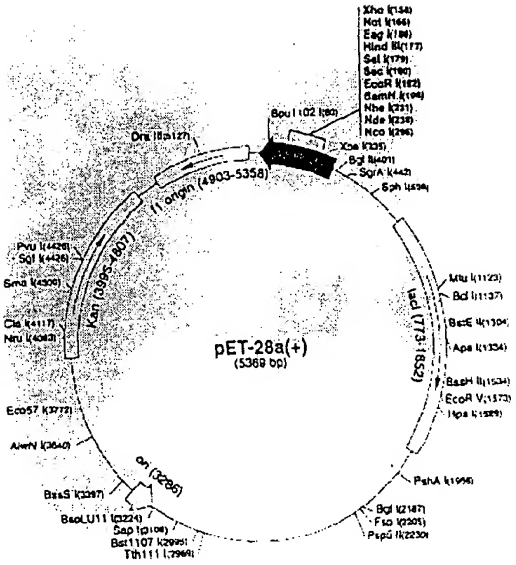



FIG. 5.0

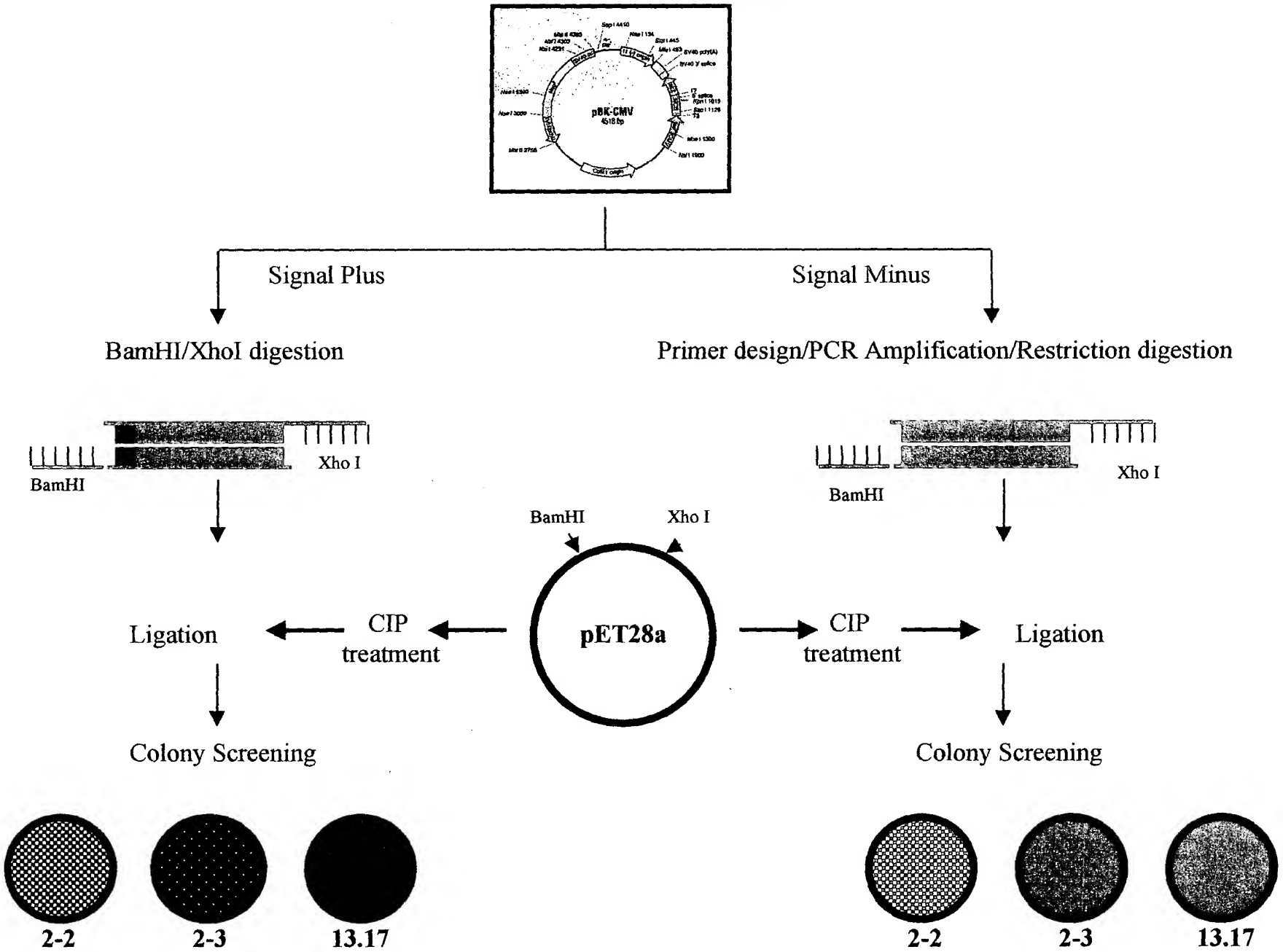

pBK-CMV
4518 bp
Signal Plus
Signal Minus
BamHI/XhoI digestion
Primer design/PCR Amplification/Restriction digestion
BamHI
Xho I
BamHI
Xho I
BamHI
Xho I
Ligation
CIP
treatment
pET28a
CIP
treatment
Ligation
Colony Screening
Colony Screening
2-2
2-3
13.17
2-2
2-3
13.17

FIG. 5.1

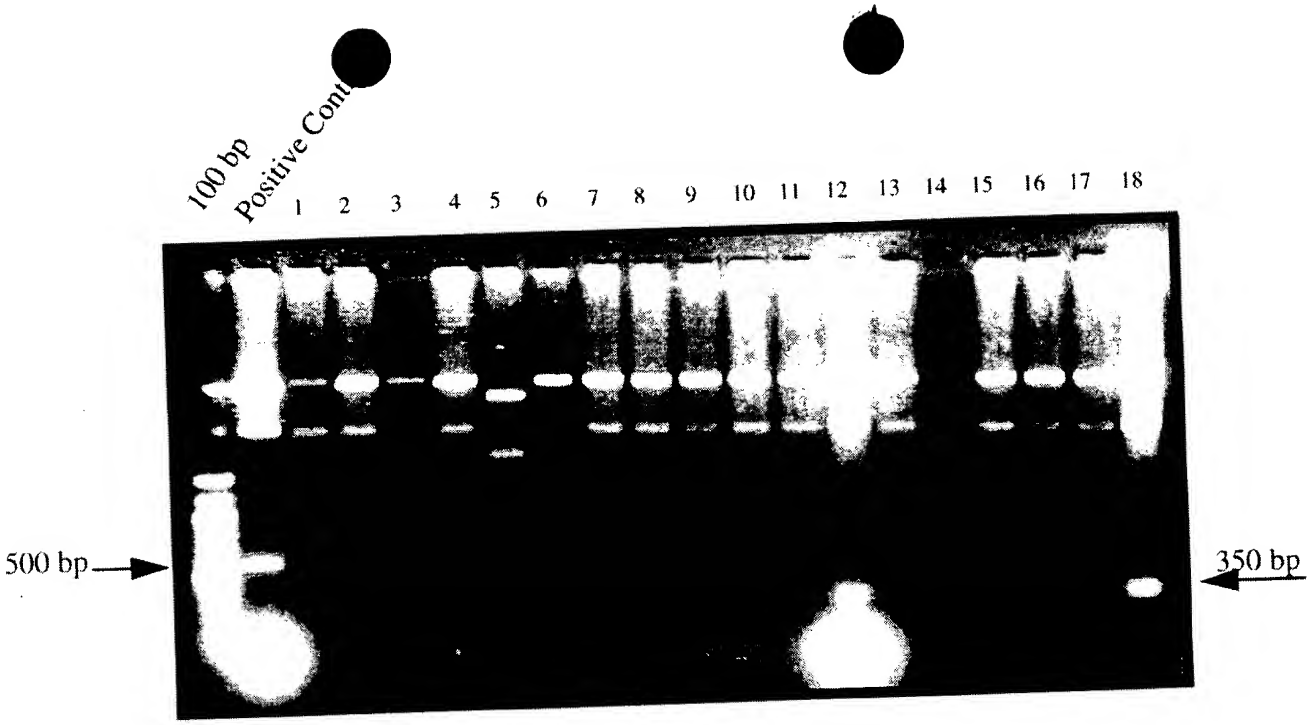



FIG. 5.2

PF-0060-0450-US

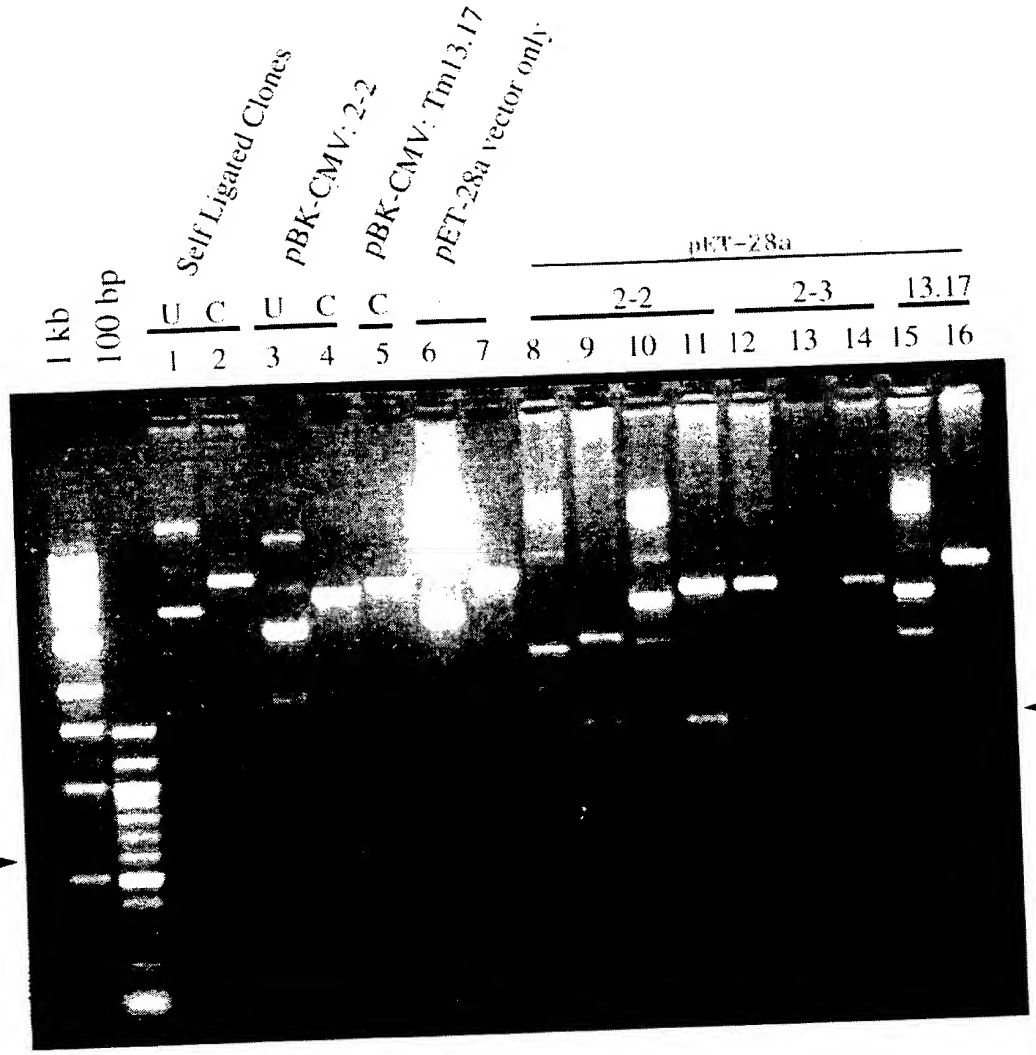



FIG. 5.3

EP 2 090 646 A2 20090819

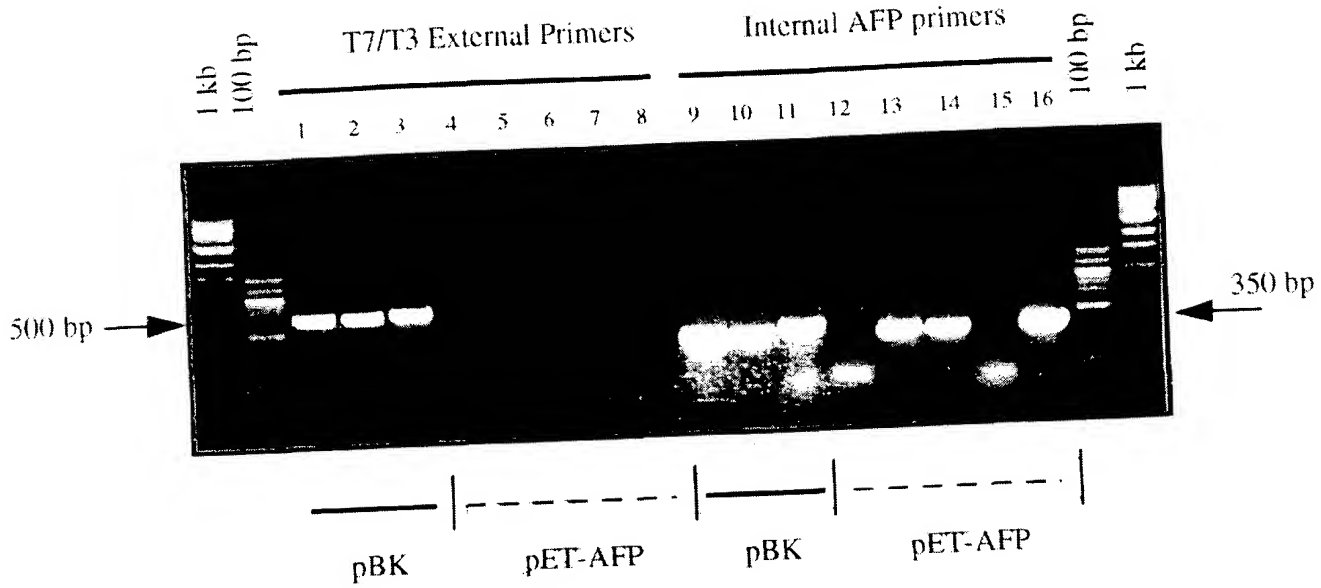



FIG. 5.4

EP 2 050 818 A1

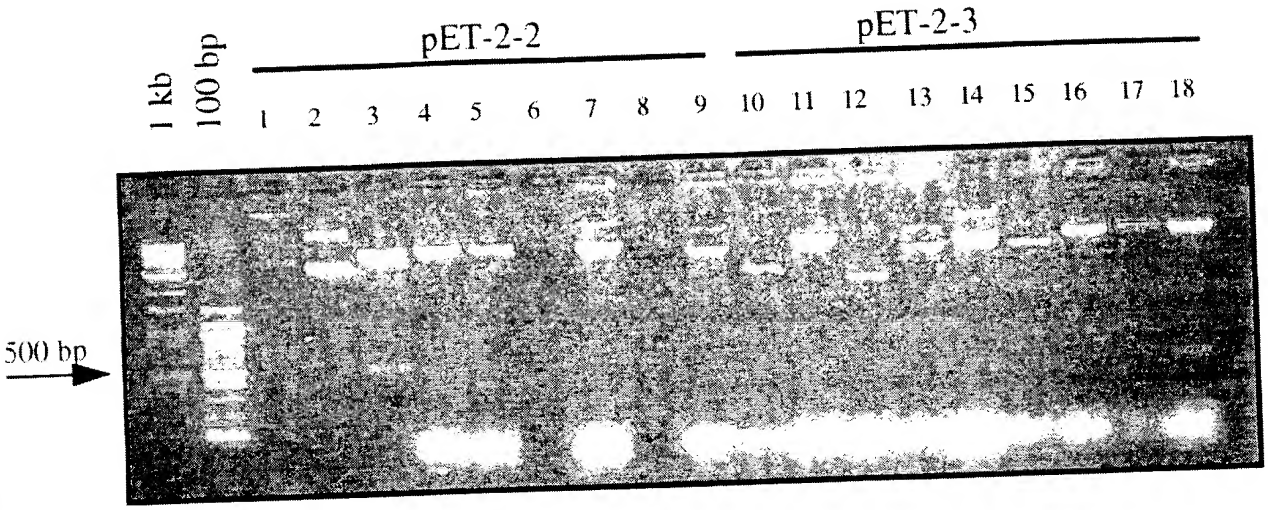

1 kb
100 bp
pET-2-2
pET-2-3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
500 bp

**FIG. 5.5**

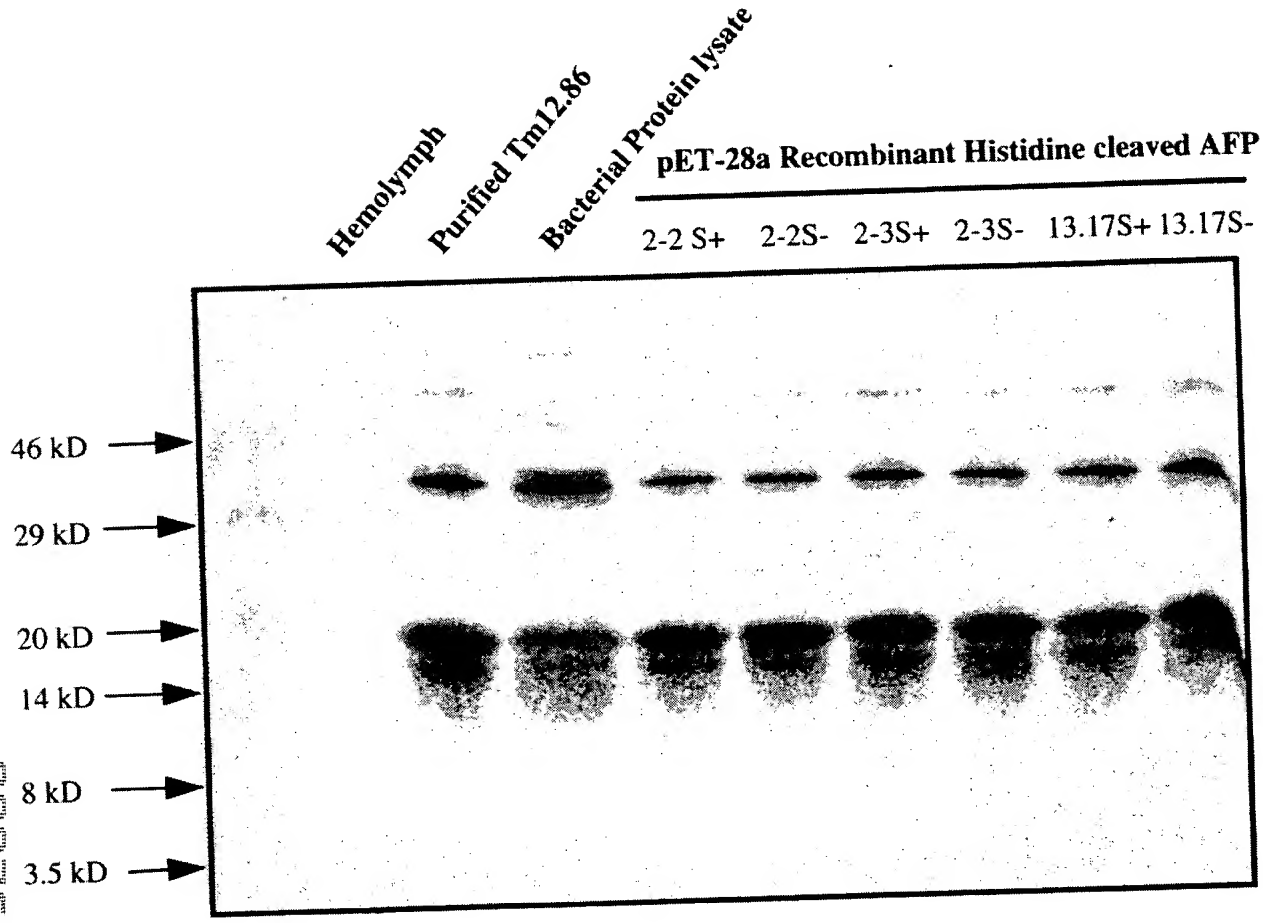



FIG. 5.6

EXHIBIT 1003 - Page 56 of 63

His-tagged Clone 2.2 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                           -55                 -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
       -45                 -40                 -35

                                                      AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG              186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
       -30                 -25                 -20

AAA CTC CTC TTG TGC TTT GCG TTC GCC GCC ATC GTC ATC GGA GCT              231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
       -15                 -10                 -5

       N-terminal of mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC              276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
       1               5                   10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC              321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG              366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC              411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG              456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC              501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT              546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                          Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA           595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                  Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTAATA TCGTTATGTA AAAAAAAAAA                  645

AAAAAACTCG AGCACCACCA CCACCACCAC TGAGAT                                 681
```

**FIG. 5.7**

His-tagged clone 2.2 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG          50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC      96
               Met Gly Ser Ser His His His His His His Ser
                                -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT    141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
           -20                 -15                 -10

                                    N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG    186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
           -5                  1                   5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG    231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
        10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT    276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
        25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT    321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
        40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA    366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
        55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAG GTG GAC AAG ATC    411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
        70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT    456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
        85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT    501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
        100                 105                 110

            Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT               543
Pro Ile Asp *
        115
```

**FIG. 5.8**

His-tagged clone 2.3 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                           -55                 -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
       -45                 -40                 -35

                                                      AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG             186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
       -30                 -25                 -20

AAA CTC CTC TTG TGC TTT GCT TTC GCC GCC ATC GTC ATC GGA GCT             231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
       -15                 -10                 -5

       N-terminal of Mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC             276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
       1                5                  10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC             321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG             366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC             411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG             456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC             501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT             546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                          Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA          595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                  Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTACTA TCGTTATGAA AAAAAAAAAA                  645

AAAAAAACTC GAGCACCACC ACCACCACCA CTGAGAT                                682
```

**FIG. 5.9**

His-tagged Clone 2.3 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG          50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC       96
               Met Gly Ser Ser His His His His His His Ser
                                -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT     141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
           -20                 -15                 -10

                                    N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG     186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
           -5                  1                   5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG     231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
        10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT     276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
        25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT     321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
        40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA     366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
        55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAA GTG GAC AAG ATC     411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
        70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT     456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
        85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT     501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
        100                 105                 110

            Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT                543
Pro Ile Asp *
        115
```

**FIG. 5.10**

US 6,756,360 B2

His-tagged Tm 13.17 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
               -65                 -60                 -55

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
               -50                 -45                 -40

GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT             186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile
               -35                 -30                 -25

                        AFP Start Codon
CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC             231
Arg His Glu Thr Thr Lys Met Lys Leu Leu Cys Cys Leu Ile Ser
               -20                 -15                 -10

                                    N-terminal of mature AFP
CTC ATT CTG TTG GTC ACA GTT CAG GCC CTG ACC GAG GCA CAA ATT             276
Leu Ile Leu Leu Val Thr Val Gln Ala Leu Thr Glu Ala Gln Ile
               -5                  1                   5

GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA             321
Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly
            10                  15                  20

GTG TCG CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG             366
Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu
            25                  30                  35

GAC GAT CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC             411
Asp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn
            40                  45                  50

GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG             456
Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu
            55                  60                  65

AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG             501
Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu
            70                  75                  80

AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG             546
Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu
            85                  90                  95

ACG GTG TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG             595
Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys
            100                 105                 110

                    Stop Codon
TTC TCA CCA GTT GAT TGA ACCACCACGA CTAGTAGATG GTTCAAATGG                643
Phe Ser Pro Val Asp *
            115

                  Polyadenylation signal  Poly-A tail
TGTGCTTTAC ATATAAAAAT AAAGTGTTTC TGATGTAAAA AAAAAAAAAA                  693

AAAAAAAAAA AACTCGAGAG TATTCTAGAG CGGCCGCGGG CCCATCGTTT                  743

TCCACCCCTC GAGCACCACC ACCACCACCA CTGAGAT                                777
```

**FIG. 5.11**

His-tagged Tm 13.17 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                           -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
        -20                 -15                 -10

                                N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA             186
Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys
        -5                  1                   5

CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG             231
Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser
    10                  15                  20

CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT             276
Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp
    25                  30                  35

CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT             321
Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly
    40                  45                  50

CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG             366
Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu
    55                  60                  65

AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC             411
Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile
    70                  75                  80

ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG             456
Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val
    85                  90                  95

TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA             501
Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser
    100                 105                 110

           Stop Codon
CCA GTT GAT TGA CTCGAGCACC ACCACCACCA CCACTGAGAT                        543
Pro Val Asp *
    115
```

**FIG. 5.12**

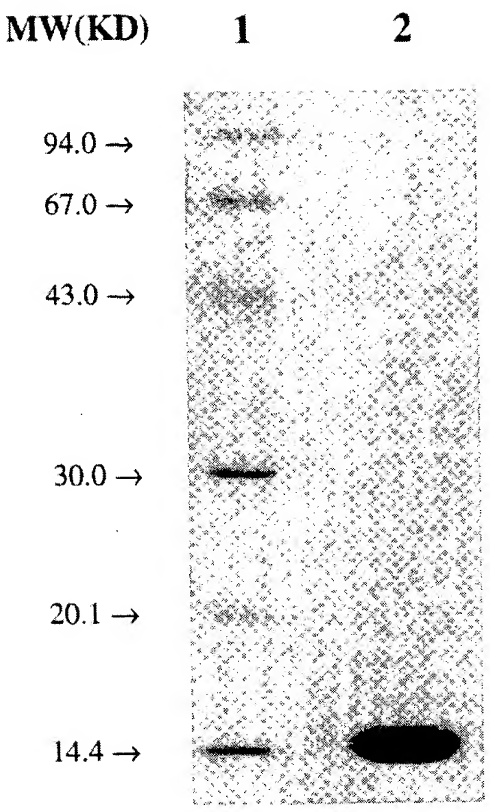



**FIG. 6.0**

EP 0 950 708 A2

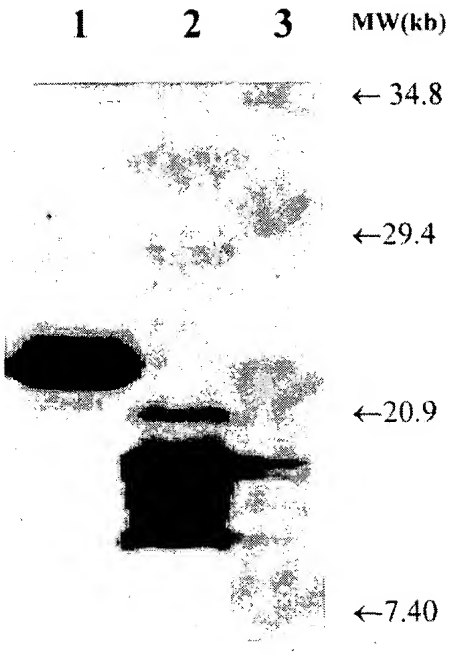



FIG. 6.1

PCT/US00/34960

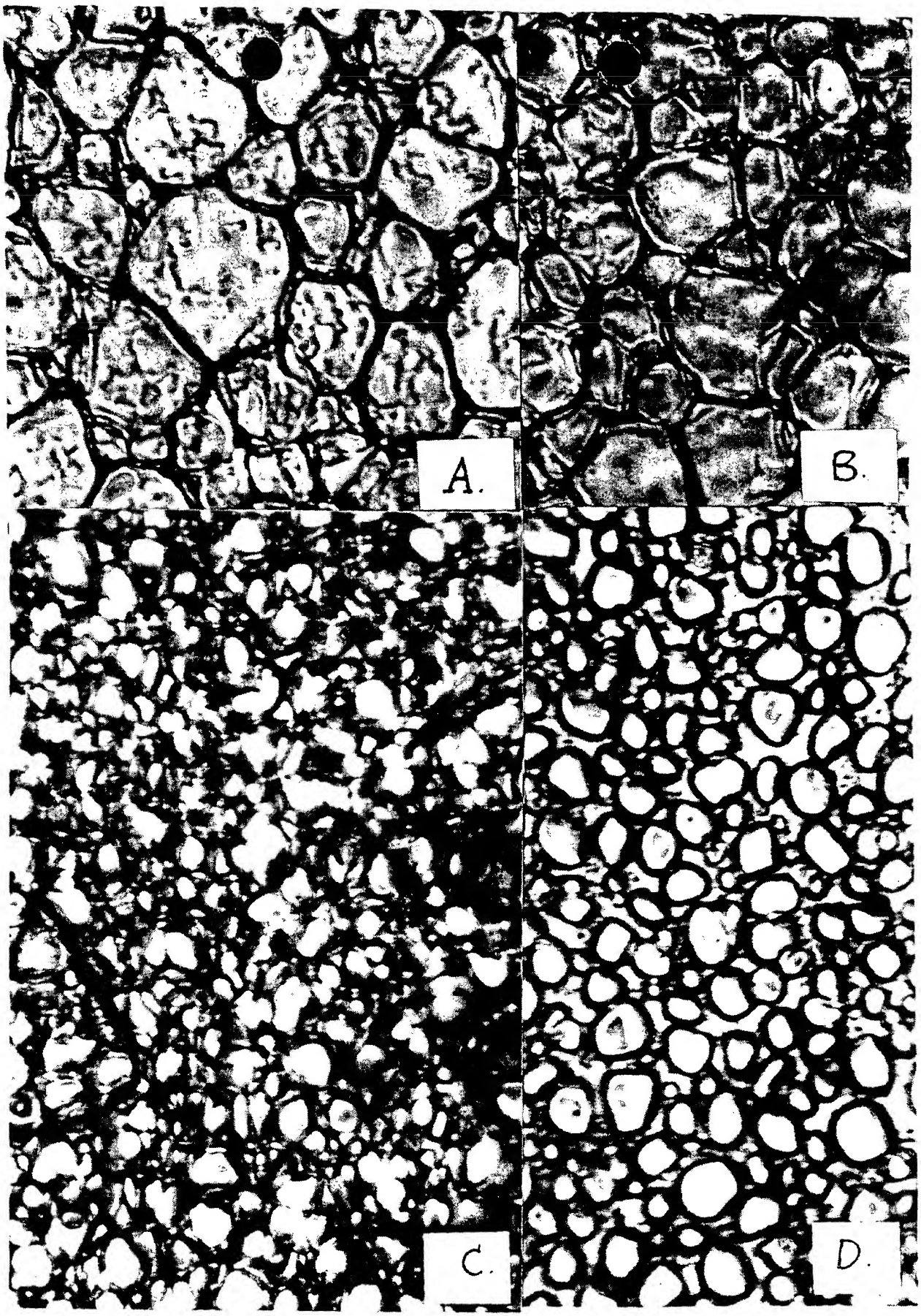



FIG. 6.2

EP 2 050 814 A2

Tm 13.17 S- graph data

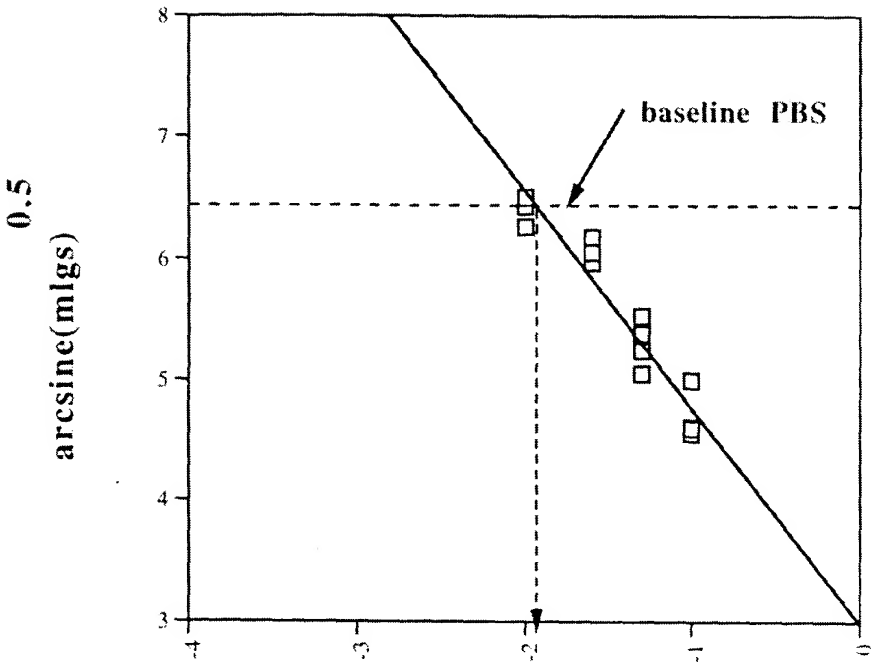



FIG. 6.3

| ONE LETTER | NAME | THREE LETTER | CHEMICAL CLASS | HYDROPHOBICITY | Reactivity |
|---|---|---|---|---|---|
| A | Alanine | Ala | aliphatic | mod. hydrophobic | low |
| B | Asp or Asn | Asx | | | |
| C | Cysteine | Cys | sulfhydryl | hydrophobic | high |
| D | Aspartic Acid | Asp | acidic | highly hydrophilic | high |
| E | Glutamic Acid | Glu | acidic | highly hydrophilic | high |
| F | Phenylalanine | Phe | aromatic | highly hydrophobic | low |
| G | Glycine | Gly | aliphatic | mod. hydrophobic | low |
| H | Histidine | His | basic, imidazole | highly hydrophilic | high |
| I | Isoleucine | Ile | aliphatic | hydrophobic | low |
| J | | | | | |
| K | Lysine | Lys | basic | highly hydophilic | high |
| L | Leucine | Leu | aliphatic | hydrophobic | low |
| M | Methionine | Met | sulfhydryl | hydrophobic | low |
| N | Asparagine | Asn | amide, acidic derived | hydrophilic | high |
| O | | | | | |
| P | Proline | Pro | aliphatic, cyclic, imino | mod. hydrophilic | low |
| Q | Glutamine | Gln | amide, acidic derived | hydrophilic | high |
| R | Arginine | Arg | basic | highly hydrophilic | high |
| S | Serine | Ser | aliphatic hydroxyl | hydrophilic | high |
| T | Threonine | Thr | aliphatic hydroxyl | hydrophilic | high |
| U | | | | | |
| V | Valine | Val | aliphatic | hydrophobic | low |
| W | Tryptophan | Trp | aromatic | highly hydrophobic | low |
| X | | | | | |
| Y | Tyrosine | Tyr | aromatic | mod. hydrophilic | high |
| Z | Glu or Gln | Glx | | | |

| | |
|---|---|
| ACD | Any Acidic |
| ALP | Any Aliphatic |
| ALH | Any Aliphatic Hydroxyl |
| ARO | Any Aromatic |
| BAS | Any Basic |
| HY- | Hydrophobic |
| HY+ | Hydrophilic |

EP 2 956 484 B1

**FIG. 7.1**

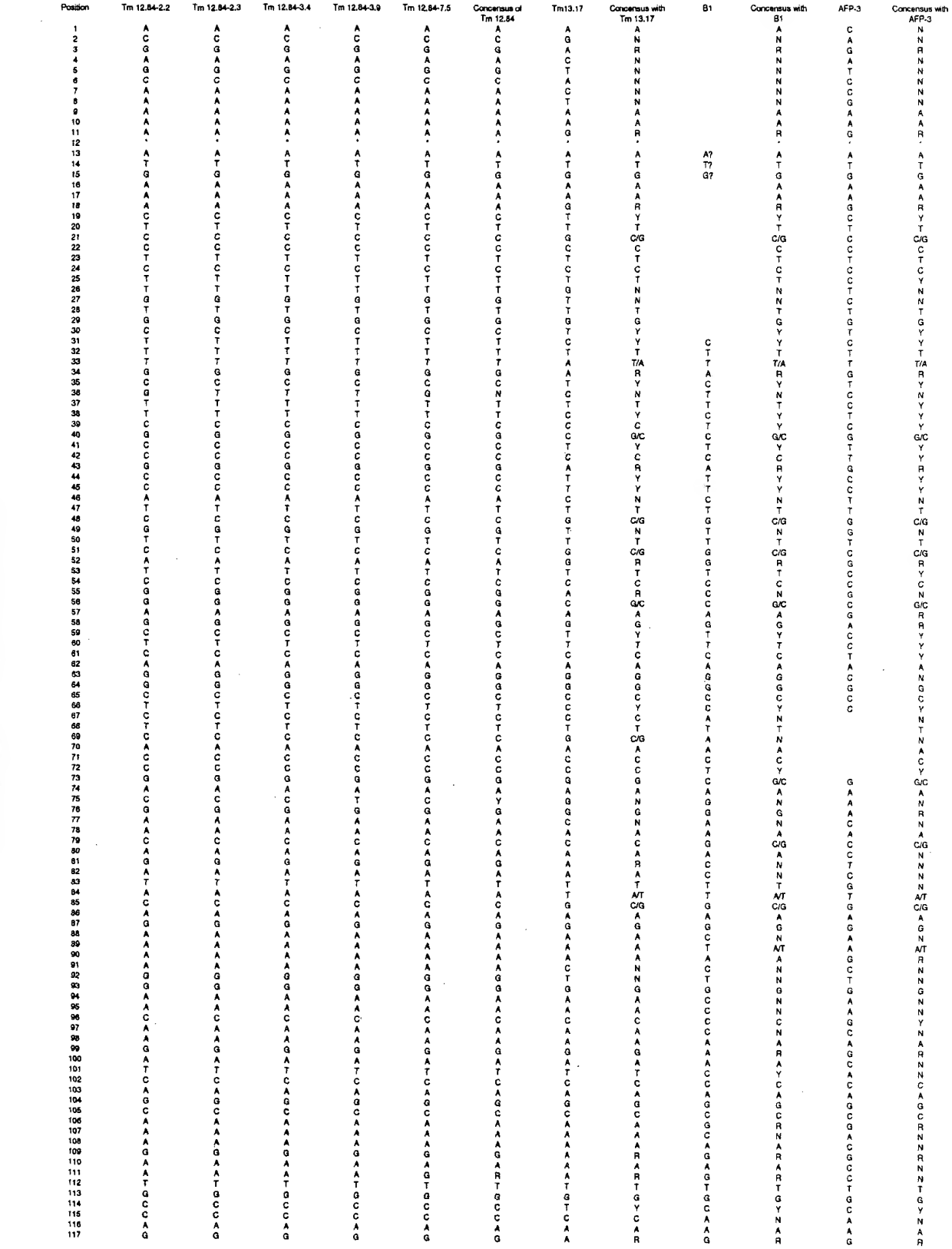

Position
Tm 12.84-2.2
Tm 12.84-2.3
Tm 12.84-3.4
Tm 12.84-3.9
Tm 12.84-7.5
Concensus of Tm 12.84
Tm13.17
Concensus with Tm 13.17
B1
Concensus with B1
AFP-3
Concensus with AFP-3

FIG. 7.2

Patent Application Publication Sep. 25, 2008 Sheet 9 of 9 US 2008/0233116 A1

| Position | Tm 12.84-2.2 | Tm 12.84-2.3 | Tm 12.84-3.4 | Tm 12.84-3.9 | Tm 12.84-7.5 | Concensus of | Tm13.17 | Concensus with | B1 | Concensus with | AFP-3 | Concensus with |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 118 | C | C | C | C | C | C | A | N | A | N | G | N |
| 119 | A | A | A | A | A | A | A | A | C | N | C | N |
| 120 | G | G | G | G | G | G | T | N | T | N | T | N |
| 121 | G | G | G | G | G | G | G | G | G | G | G | G |
| 122 | T | T | T | A | T | T/A | A | T/A | A | T/A | A | A/T |
| 123 | G | T | G | G | G | G | A | R | A | R | G | R |
| 124 | T | T | T | T | T | T | A | T/A | A | T/A | T | T/A |
| 125 | C | C | C | C | C | C | G | C/G | G | C/G | C | C/G |
| 126 | C | C | C | C | C | C | T | Y | T | Y | C | Y |
| 127 | G | G | G | G | G | G | G | G | G | G | G | G |
| 128 | G | G | G | G | G | G | G | G | G | G | G | G |
| 129 | A | A | A | A | A | A | A | A | A | A | A | A |
| 130 | G | T | G | T | G | G | A | G | G | G | G | G |
| 131 | T | T | T | T | T | T | G | T | T | T | T | T |
| 132 | G | T | G | T | G | T | G | T | G | T | A | R |
| 133 | T | T | T | T | T | T | T | C | T | C | T | T |
| 134 | C | C | C | C | C | C | C | C | C | C | C | C |
| 135 | C | C | C | C | C | C | G | C/G | C | C/G | C | N |
| 136 | C | C | C | C | C | C | C | C | G | C/G | G | C/G |
| 137 | A | A | A | A | A | A | A | A | A | A | A | A |
| 138 | A | A | A | A | A | A | A | A | A | A | A | A |
| 139 | G | G | G | G | G | G | G | G | A | G | A | G |
| 140 | A | A | A | A | A | A | A | A | G | A | A | A |
| 141 | G | G | G | G | G | G | G | G | C | G/C | G | G/C |
| 142 | A | A | A | A | A | A | A | A | G | R | T | N |
| 143 | C | C | C | C | C | C | T | Y | T | Y | C | Y |
| 144 | G | G | G | G | G | G | C | G/C | C | G/C | C | G/C |
| 145 | A | A | A | A | A | A | A | A | A | A | C | N |
| 146 | T | T | T | T | T | T | A | T | A | T | C | T |
| 147 | C | C | C | C | C | C | T | N | A | N | C | N |
| 148 | G | G | G | G | G | G | A | R | A | R | A | R |
| 149 | A | A | A | A | A | A | C | N | G | N | A | N |
| 150 | C | C | C | C | C | C | C | C | A | C/G | C | C/G |
| 151 | A | A | A | A | A | A | A | A | G | A | A | A |
| 152 | A | A | A | A | A | A | A | A | A | R | A | R |
| 153 | A | A | A | A | A | A | A | A | G | R | G | R |
| 154 | G | G | G | G | G | G | G | G | C | Y | G | Y |
| 155 | T | T | T | T | T | T | C | Y | T | Y | T | Y |
| 156 | C | C | C | C | C | C | T | Y | C | Y | C | Y |
| 157 | C | C | C | C | C | C | C | C | G | C | G | C |
| 158 | G | G | G | G | G | G | G | G | C | G | C | G |
| 159 | C | C | C | C | C | C | C | C | A | C | A | C |
| 160 | A | A | A | A | A | A | A | A | N | A | A | A |
| 161 | C | C | C | C | C | C | A | A | N | A | A | N |
| 162 | A | A | A | A | A | A | C | N | G | N | C | N |
| 163 | G | G | G | G | G | G | G | G | G | G | C | G/C |
| 164 | G | G | G | G | G | G | G | G | G | G | G | G |
| 165 | T | T | T | T | T | T | G | T | G | T | T | T |
| 166 | G | T | G | T | G | T | G | T | G | T | G | G |
| 167 | T | T | T | T | T | T | A | T/A | A | T/A | A | T/A |
| 168 | C | C | C | C | C | C | C | C | C | C | A | N |
| 169 | T | T | T | T | T | T | T | T | T | T | G | N |
| 170 | T | T | T | T | T | T | G | N | G | N | A | R |
| 171 | G | G | G | G | G | G | G | G | G | G | A | G |
| 172 | G | G | G | G | G | G | G | G | A | T/A | G | T/A |
| 173 | T | T | T | T | T | T | A | T/A | G | C/G | T | C/G |
| 174 | C | C | C | C | C | C | G | C/G | G | G | G | G |
| 175 | G | G | G | G | G | G | G | G | A | A | A | A |
| 176 | A | A | A | A | A | A | A | A | C | Y | C | Y |
| 177 | C | C | C | C | C | C | G | Y | G | G | G | G |
| 178 | G | G | G | G | G | G | A | G | A | A | A | A |
| 179 | A | A | A | A | A | A | T | A | T | T | T | T |
| 180 | T | T | T | T | T | T | C | T | C | C | C | C |
| 181 | C | C | C | C | C | C | C | C | C | C | C | C |
| 182 | C | C | C | C | C | C | C | C | C | C | C | C |
| 183 | C | C | C | C | C | C | C | C | C | C | C | C |
| 184 | A | A | A | A | A | A | A | A | C | Y | C | Y |
| 185 | A | A | A | A | A | A | A | A | A | A | A | A |
| 186 | A | A | A | A | A | A | A | A | A | A | A | A |
| 187 | A | A | A | A | A | A | C | N | A | A | A | A |
| 188 | T | T | T | T | T | T | T | T | C | T | C | T |
| 189 | G | G | G | G | G | G | G | G | T | G | T | G |
| 190 | A | A | A | A | A | A | A | A | G | A | G | A |
| 191 | A | A | A | A | A | A | A | A | A | A | A | A |
| 192 | G | G | G | G | G | G | A | G | A | A | A | A |
| 193 | A | A | A | A | A | A | C | N | A | A | A | A |
| 194 | A | A | A | A | A | A | G | A | T | R | A | R |
| 195 | G | G | G | G | G | G | C | G/C | G | N | G | N |
| 196 | C | C | C | C | C | C | C | C | C | C | C | C |
| 197 | A | A | A | A | A | A | A | A | A | A | A | A |
| 198 | C | C | C | C | C | C | A | N | A | A | A | N |
| 199 | G | G | G | G | G | G | G | G | C | G/C | T | G/C |
| 200 | T | T | T | T | T | T | T | T | T | T | C | Y |
| 201 | C | C | C | C | C | C | T | Y | T | Y | T | Y |
| 202 | C | C | C | C | C | C | T | Y | T | Y | T | Y |
| 203 | T | T | T | T | T | T | T | T | T | T | C | T |
| 204 | C | C | C | C | C | C | T | Y | T | Y | T | Y |
| 205 | T | T | T | T | T | T | G | T | G | G | G | G |
| 206 | G | G | G | G | G | G | C | G | C | C | C | C |
| 207 | C | C | C | C | C | C | T | C | N | T | A | N |
| 208 | T | T | T | T | T | T | C | T | T | C | T | T |
| 209 | T | T | T | T | T | T | G | G | T | G | C | G |
| 210 | C | C | C | C | C | C | C | C/G | T | C/G | T | C/G |
| 211 | T | T | T | T | T | T | G | N | T | N | C | N |
| 212 | C | C | C | C | C | C | C | C | T | C | T | C |
| 213 | G | G | G | G | G | G | C | G/C | T | C | T | C |
| 214 | A | A | A | A | A | A | C | A | A | A | G | A |
| 215 | A | A | A | A | A | A | A | R | A | A | A | R |
| 216 | G | G | G | G | G | G | A | G | A | A | A | G |
| 217 | A | A | A | A | A | A | A | A | G | G | A | R |
| 218 | A | A | A | A | A | A | A | A | C | R | G | R |
| 219 | A | A | A | A | A | A | A | A | A | N | A | N |
| 220 | A | A | A | A | A | A | A | A | C | N | A | N |
| 221 | C | C | C | C | C | C | A | A | T | C | A | Y |
| 222 | T | T | T | T | T | T | C | T | C | Y | G | Y |
| 223 | G | G | G | G | G | G | G | G | C | G | G | G |
| 224 | G | G | G | G | G | G | G | G | T | G | G | R |
| 225 | A | A | A | A | A | A | G | A | C | A/T | A | A/T |
| 226 | G | G | G | G | G | G | T | G/C | T | N | T | N |
| 227 | T | T | T | T | T | T | G | T | A | T | T | T |
| 228 | G | G | G | G | G | G | G | G | T | G | T | N |
| 229 | G | G | G | G | G | G | G | G | A | G | A | R |
| 230 | C | C | C | C | C | C | C | C | G | Y | T | Y |
| 231 | A | A | A | A | A | A | A | N | T | N | C | N |
| 232 | A | A | A | A | A | A | A | A | C | R | G | R |
| 233 | C | C | C | C | C | C | C | C | G | C | A | N |
| 234 | C | C | C | C | C | C | G | C/G | G | C/G | C | C/G |
| 235 | G | G | G | G | G | G | G | G | G | G | G | G |

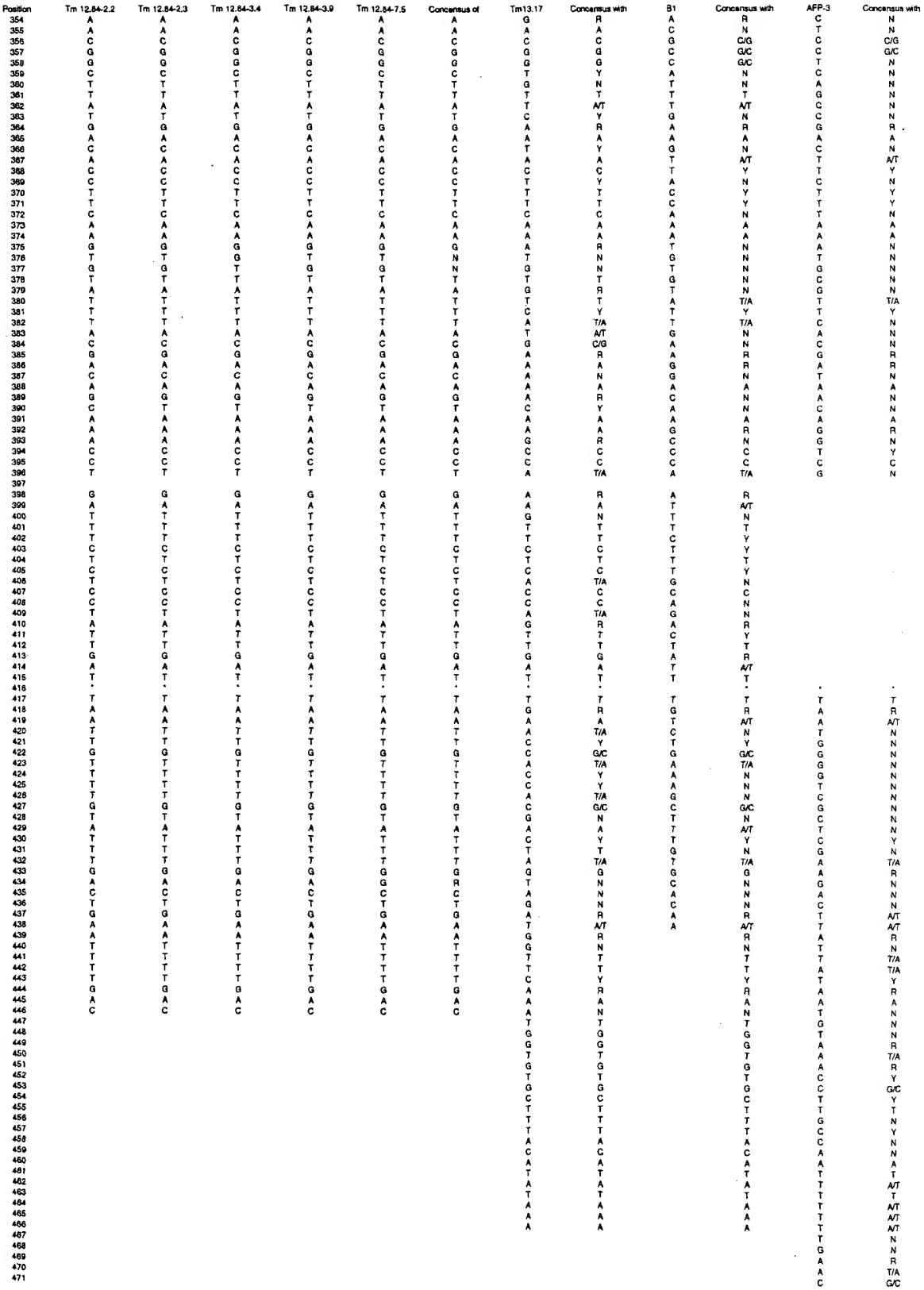
FIG. 7.2 Cont.

10/20/30 "Bhe52/850

| Position | Tm 12.84-2.2 | Tm 12.84-2.3 | Tm 12.84-3.4 | Tm 12.84-3.9 | Tm 12.84-7.5 | Concensus of | Tm13.17 | Concensus with | S1 | Concensus with | AFP-3 | Concensus with |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 236 | A | A | A | A | A | A | A | A | A | A | C | N |
| 237 | A | A | A | A | A | A | A | A | A | A | C | N |
| 238 | G | G | C | G | G | G | T | N | T | N | A | N |
| 239 | C | C | C | C | C | C | C | C | C | C | G | C/G |
| 240 | C | C | C | C | C | C | G | C/G | G | C/G | T | N |
| 241 | G | G | G | G | G | G | G | G | G | G | G | G |
| 242 | G | G | G | G | G | G | G | G | G | G | G | G |
| 243 | A | A | A | A | A | A | A | A | A | A | T | A/T |
| 244 | G | G | G | G | G | G | G | G | G | G | G | G |
| 245 | A | A | A | A | A | A | A | A | A | A | A | A |
| 246 | C | A | C | A | C | A | G | C/G | A | N | A | N |
| 247 | A | A | A | A | A | A | G | R | A | R | T | N |
| 248 | C | C | C | C | C | C | T | Y | T | Y | T | Y |
| 249 | C | C | C | C | C | C | G | C/G | T | N | T | N |
| 250 | A | A | A | A | A | A | G | R | G | R | C | N |
| 251 | A | T | A | T | T | A | T | A/T | A | A/T | A | A/T |
| 252 | T | T | T | T | T | T | G | N | G | N | A | N |
| 253 | G | T | G | T | G | T | G | G | G | G | C | G/C |
| 254 | T | T | T | T | T | T | T | T | C | Y | T | Y |
| 255 | G | G | G | G | G | G | C | G/C | C | G/C | G | G/C |
| 256 | G | G | G | G | G | G | G | G | G | G | G | G |
| 257 | A | A | A | A | A | A | A | A | A | A | A | A |
| 258 | G | A | G | G | G | G | C | G/C | C | G/C | C | G/C |
| 259 | G | G | G | G | G | G | G | G | A | R | C | N |
| 260 | T | A | T | T | A | T | G | T | C | Y | A | N |
| 261 | A | A | A | A | A | A | G | R | G | R | C | N |
| 262 | C | T | C | T | C | C | T | Y | T | Y | A | N |
| 263 | T | T | T | T | T | T | T | T | T | T | T | T |
| 264 | C | C | C | C | C | C | G | C/G | C | C/G | T | N |
| 265 | A | A | A | A | A | A | A | A | A | A | A | A |
| 266 | A | A | A | A | A | A | G | R | A | R | A | R |
| 267 | A | A | A | A | A | A | G | R | G | R | G | R |
| 268 | G | G | G | G | G | G | G | G | G | G | A | R |
| 269 | C | C | C | C | C | C | A | N | A | N | C | N |
| 270 | C | A | C | A | C | C | G | C/G | G | C/G | G | C/G |
| 271 | A | A | A | A | A | A | A | A | A | A | A | A |
| 272 | A | A | A | A | A | A | A | A | A | A | A | A |
| 273 | G | G | G | G | G | G | G | G | G | G | A | R |
| 274 | C | C | C | C | C | C | G | C/G | T | N | T | N |
| 275 | T | T | T | T | T | T | T | T | T | T | T | T |
| 276 | G | A | G | A | G | G | G | G | G | G | C | G/C |
| 277 | A | A | A | A | A | A | A | A | A | A | A | A |
| 278 | A | A | A | A | A | A | G | R | C | N | A | N |
| 279 | G | C | G | C | G | G | G | G | G | G | G | G |
| 280 | C | A | C | A | C | C | A | N | A | N | G | N |
| 281 | A | T | A | T | A | A | A | A | G | R | A | R |
| 282 | T | T | T | T | T | T | G | N | G | N | A | N |
| 283 | T | T | T | T | T | T | G | T | G | N | A | N |
| 284 | T | T | T | T | T | T | T | T | G | N | A | N |
| 285 | T | G | T | G | T | T | C | G/C | A | N | C | N |
| 286 | G | G | G | G | G | G | A | R | C | N | T | N |
| 287 | C | C | C | C | C | C | C | C | A | N | C | N |
| 288 | C | C | C | C | C | C | A | Y | A | N | T | N |
| 289 | A | A | A | A | A | A | G | R | A | R | G | R |
| 290 | G | G | G | G | G | G | A | R | C | N | A | N |
| 291 | C | C | C | C | C | C | C | C | G | C/G | G | C/G |
| 292 | | | | | | | A | A | A | A | G | R |
| 293 | | | | | | | A | A | T | A/T | A | A/T |
| 294 | | | | | | | C | C/G | G | C/G | T | N |
| 295 | G | G | G | G | G | G | G | G | A | R | T | N |
| 296 | A | A | A | A | A | A | A | A | T | A/T | C | N |
| 297 | C | C | C | C | C | C | C | C | G | C/G | G | C/G |
| 298 | G | A | G | G | G | G | G | G | A | R | G | R |
| 299 | A | A | A | A | A | A | A | A | A | A | A | A |
| 300 | A | A | A | A | A | A | A | A | G | R | A | R |
| 301 | G | G | G | G | G | G | G | G | A | R | A | R |
| 302 | A | A | A | A | A | A | A | A | A | A | G | A |
| 303 | G | A | G | A | G | G | A | R | A | R | G | R |
| 304 | G | T | G | T | G | G | T | R | G | R | T | R |
| 305 | T | T | T | T | T | T | C | Y | G | C | T | Y |
| 306 | T | G | T | G | T | G | T | G | G | N | C | N |
| 307 | G | G | G | A | G | G | G | G | A | R | G | R |
| 308 | A | A | A | A | A | A | G | A | G | R | A | R |
| 309 | C | A | C | A | C | C | A | C/G | A | N | T | N |
| 310 | A | A | A | A | A | A | A | A | A | A | G | R |
| 311 | A | A | A | A | A | A | A | A | G | R | A | R |
| 312 | G | A | G | A | G | G | A | R | A | R | C | N |
| 313 | A | T | A | T | A | A | T | A/T | T | A/T | T | A/T |
| 314 | T | C | T | C | T | T | C | T | T | T | T | T |
| 315 | C | G | C | G | C | C | T | C/G | G | C/G | G | C/G |
| 316 | G | T | G | T | G | G | C | R | T | N | G | N |
| 317 | T | T | T | G | T | T | T | T | C | Y | T | Y |
| 318 | G | G | G | G | G | G | C | G/C | G | G/C | T | N |
| 319 | C | C | C | C | C | C | A | N | A | N | G | N |
| 320 | A | A | A | A | A | A | A | A | G | R | C | N |
| 321 | G | G | G | A | G | G | T | N | A | N | C | N |
| 322 | A | A | A | A | A | A | A | A | A | A | A | A |
| 323 | A | A | A | A | A | A | A | A | G | R | A | R |
| 324 | G | T | G | T | G | G | T | G | T | N | A | N |
| 325 | T | T | T | T | T | T | G | T | G | N | T | N |
| 326 | G | G | G | G | G | G | T | G | C | G/C | G | G/C |
| 327 | C | C | C | C | C | C | C | C | A | N | T | N |
| 328 | G | G | G | G | G | G | G | G | C | G/C | G | G/C |
| 329 | T | T | T | T | T | T | C | Y | G | N | C | N |
| 330 | G | G | G | G | G | G | C | G/C | G | G/C | C | G/C |
| 331 | G | T | G | G | G | G | G | G | T | N | G | N |
| 332 | T | T | T | T | T | T | T | T | T | N | T | N |
| 333 | C | C | C | C | C | C | C | C | A | N | C | N |
| 334 | A | A | A | A | A | A | A | A | C | N | A | N |
| 335 | A | A | A | A | A | A | A | A | T | N | A | N |
| 336 | G | G | G | G | G | G | G | G | G | G | G | G |
| 337 | A | A | A | A | A | A | A | A | A | A | A | A |
| 338 | A | A | A | A | A | A | G | R | A | R | A | R |
| 339 | G | G | G | G | G | G | G | R | C | N | G | N |
| 340 | G | G | G | G | G | G | G | G | A | R | G | R |
| 341 | C | C | C | C | C | C | C | C | C | N | A | N |
| 342 | C | C | C | C | C | C | A | Y | A | N | C | N |
| 343 | A | C | A | C | A | A | C | A | C | N | A | N |
| 344 | C | C | C | C | C | C | C | C | T | Y | C | Y |
| 345 | A | C | A | C | A | A | C | A/T | C | N | T | N |
| 346 | C | C | C | C | C | C | C | C/G | G | C/G | C | C/G |
| 347 | C | C | C | C | C | C | C | C | G | N | C | N |
| 348 | A | A | A | A | A | A | A | A/T | G | N | T | N |
| 349 | G | A | G | A | G | G | A | G | A | R | C | N |
| 350 | A | A | A | A | A | A | A | A | A | A | A | A |
| 351 | G | G | G | G | G | G | G | R | G | R | G | R |
| 352 | G | G | G | A | G | G | G | G | A | R | C | N |
| 353 | A | A | A | A | A | A | A | A | T | A/T | A | A/T |

FIG. 7.2 Cont.

PCT/US98/24360



| Position | Tm 12.84-2.2 | Tm 12.84-2.3 | Tm 12.84-3.4 | Tm 12.84-3.9 | Tm 12.84-7.5 | Concensus of | Tm13.17 | Concensus with | B1 | Concensus with | AFP-3 | Concensus with |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 354 | A | A | A | A | A | A | G | R | A | R | C | N |
| 355 | A | A | A | A | A | A | A | A | C | N | T | N |
| 356 | C | C | C | C | C | C | C | C | G | C/G | C | C/G |
| 357 | G | G | G | G | G | G | G | G | C | G/C | C | G/C |
| 358 | G | G | G | G | G | G | G | G | C | G/C | T | N |
| 359 | C | C | C | T | C | C | G | Y | A | N | C | N |
| 360 | T | T | T | T | T | T | T | N | T | N | A | N |
| 361 | T | T | T | T | T | T | T | T | T | T | G | N |
| 362 | A | A | A | A | A | A | T | A/T | T | A/T | C | N |
| 363 | T | T | T | T | T | T | C | Y | G | N | C | N |
| 364 | G | G | G | G | G | G | A | R | A | R | G | R |
| 365 | A | A | A | A | A | A | A | A | G | A | A | A |
| 366 | C | C | C | C | C | C | T | Y | T | N | C | N |
| 367 | A | A | A | A | A | A | A | A | A | A | T | A/T |
| 368 | C | C | C | C | C | C | C | C | T | Y | T | Y |
| 369 | C | C | C | C | C | C | T | Y | A | N | C | N |
| 370 | T | T | T | T | T | T | T | Y | C | Y | T | Y |
| 371 | T | T | T | T | T | T | T | T | C | Y | T | Y |
| 372 | C | C | C | C | C | C | C | C | A | N | T | N |
| 373 | A | A | A | A | A | A | A | A | A | A | A | A |
| 374 | A | A | A | A | A | A | A | A | A | A | A | A |
| 375 | G | G | G | G | G | G | A | R | G | A | T | N |
| 376 | T | T | T | T | T | T | T | N | T | N | G | N |
| 377 | T | T | T | T | T | T | T | N | T | N | C | N |
| 378 | T | T | T | T | T | T | T | T | T | N | G | N |
| 379 | A | A | A | A | A | A | T | R | T | N | C | N |
| 380 | T | T | T | T | T | T | T | T | A | T/A | T | T/A |
| 381 | T | T | T | T | T | T | C | Y | T | Y | T | Y |
| 382 | T | T | T | T | T | T | A | T/A | T | T/A | C | N |
| 383 | A | A | A | A | A | A | T | A/T | G | N | A | N |
| 384 | C | C | C | C | C | C | G | C/G | A | N | C | N |
| 385 | G | G | G | G | G | G | A | R | A | R | G | R |
| 386 | A | A | A | A | A | A | A | A | G | R | A | R |
| 387 | C | C | C | C | C | C | A | A | G | R | T | N |
| 388 | A | A | A | A | A | A | A | A | A | A | A | A |
| 389 | G | G | G | G | G | G | A | R | C | N | A | N |
| 390 | C | C | C | C | C | C | C | Y | A | N | C | N |
| 391 | A | A | A | A | A | A | A | A | A | A | A | A |
| 392 | A | A | A | A | A | A | A | A | G | R | G | R |
| 393 | A | A | A | A | A | A | G | R | C | N | G | N |
| 394 | C | C | C | C | C | C | C | C | C | C | T | Y |
| 395 | C | C | C | C | C | C | C | C | C | C | C | C |
| 396 | T | T | T | T | T | T | A | T/A | A | T/A | G | N |
| 397 | | | | | | | | | | | | |
| 398 | G | G | G | G | G | G | A | R | A | R | | |
| 399 | A | A | A | A | A | A | A | A | T | A/T | | |
| 400 | T | T | T | T | T | T | G | N | T | N | | |
| 401 | T | T | T | T | T | T | T | T | T | T | | |
| 402 | T | T | T | T | T | T | T | T | C | Y | | |
| 403 | C | C | C | C | C | C | C | C | T | Y | | |
| 404 | T | T | T | T | T | T | T | T | T | T | | |
| 405 | C | C | C | C | C | C | C | C | T | Y | | |
| 406 | T | T | T | T | T | T | C | T/A | G | N | | |
| 407 | C | C | C | C | C | C | C | C | C | N | | |
| 408 | C | C | C | C | C | C | C | C | A | N | | |
| 409 | T | T | T | T | T | T | A | T/A | G | N | | |
| 410 | A | A | A | A | A | A | G | R | A | R | | |
| 411 | T | T | T | T | T | T | T | T | C | Y | | |
| 412 | T | T | T | T | T | T | T | T | T | T | | |
| 413 | G | G | G | G | G | G | A | R | A | R | | |
| 414 | A | A | A | A | A | A | T | A/T | T | A/T | | |
| 415 | T | T | T | T | T | T | T | T | T | T | | |
| 416 | · | · | · | · | · | · | · | · | · | · | · | · |
| 417 | T | T | T | T | T | T | T | T | T | T | T | T |
| 418 | A | A | A | A | A | A | G | R | G | R | A | R |
| 419 | A | A | A | A | A | A | A | A | T | A/T | A | A/T |
| 420 | T | T | T | T | T | T | C | T/A | C | N | T | N |
| 421 | T | T | T | T | T | T | C | Y | T | Y | G | N |
| 422 | G | G | G | G | G | G | C | G/C | G | G/C | G | N |
| 423 | T | T | T | T | T | T | A | T/A | A | T/A | G | N |
| 424 | T | T | T | T | T | T | C | Y | A | N | G | N |
| 425 | T | T | T | T | T | T | C | Y | A | N | T | N |
| 426 | T | T | T | T | T | T | A | T/A | G | N | C | N |
| 427 | G | G | G | G | G | G | C | G/C | C | G/C | G | N |
| 428 | T | T | T | T | T | T | G | N | T | N | C | N |
| 429 | A | A | A | A | A | A | A | A | T | A/T | T | N |
| 430 | T | T | T | T | T | T | C | Y | T | Y | C | N |
| 431 | T | T | T | T | T | T | T | T | G | N | G | N |
| 432 | T | T | T | T | T | T | A | T/A | ? | T/A | A | T/A |
| 433 | G | G | G | G | G | G | G | G | G | G | A | R |
| 434 | A | A | A | A | A | A | T | A/T | C | N | G | N |
| 435 | C | C | C | C | C | C | A | A/C | A | N | A | N |
| 436 | T | T | T | T | T | T | G | N | A | N | C | N |
| 437 | G | G | G | G | G | G | A | R | A | R | T | A/T |
| 438 | A | A | A | A | A | A | T | A/T | | A/T | T | A/T |
| 439 | A | A | A | A | A | A | G | R | | R | A | R |
| 440 | T | T | T | T | T | T | T | N | | N | T | N |
| 441 | T | T | T | T | T | T | T | T | | T | T | T/A |
| 442 | T | T | T | T | T | T | T | T | | T | A | T/A |
| 443 | T | T | T | T | T | T | T | T | | Y | T | Y |
| 444 | G | G | G | G | G | G | T | R | | R | A | R |
| 445 | A | A | A | A | A | A | C | A | | A | T | A |
| 446 | C | C | C | C | C | C | A | N | | N | G | N |
| 447 | | | | | | | A | T | | T | T | N |
| 448 | | | | | | | T | G | | G | A | N |
| 449 | | | | | | | G | T | | G | A | R |
| 450 | | | | | | | T | G | | T | C | T/A |
| 451 | | | | | | | G | T | | T | C | R |
| 452 | | | | | | | T | G | | G | T | Y |
| 453 | | | | | | | T | G | | T | T | G/C |
| 454 | | | | | | | G | C | | C | G | Y |
| 455 | | | | | | | C | T | | T | G | T |
| 456 | | | | | | | T | T | | T | C | N |
| 457 | | | | | | | T | T | | T | C | Y |
| 458 | | | | | | | T | T | | T | A | N |
| 459 | | | | | | | A | A | | A | C | N |
| 460 | | | | | | | C | A | | A | C | A |
| 461 | | | | | | | A | T | | T | A | T |
| 462 | | | | | | | T | A | | A | T | A/T |
| 463 | | | | | | | A | T | | T | T | T |
| 464 | | | | | | | T | A | | A | T | A/T |
| 465 | | | | | | | A | A | | A | T | A/T |
| 466 | | | | | | | A | A | | A | T | A/T |
| 467 | | | | | | | | | | | T | N |
| 468 | | | | | | | | | | | G | N |
| 469 | | | | | | | | | | | A | R |
| 470 | | | | | | | | | | | A | T/A |
| 471 | | | | | | | | | | | C | G/C |

FIG. 7.2 Cont.

| Position | Tm 12.84-2.2 | Tm 12.84-2.3 | Tm 12.84-3.4 | Tm 12.84-3.9 | Tm 12.84-7.5 | Concensus of | Tm13.17 | Concensus with | B1 | Concensus with | AFP-3 | Concensus with |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 472 | A | A | A | A | A | A | A | A | | A | A | A |
| 473 | A | A | A | A | A | A | A | A | | A | A | A |
| 474 | T | T | T | T | T | T | T | T | | T | T | T |
| 475 | A | A | A | A | A | A | A | A | | A | A | A |
| 476 | A | A | A | A | A | A | A | A | | A | A | A |
| 477 | A | A | A | A | A | A | A | A | | A | A | A |
| 478 | G | G | G | G | G | G | G | G | | G | T | N |
| 479 | G | G | G | G | G | G | T | N | | N | T | N |
| 480 | T | T | T | T | T | T | G | N | | N | T | N |
| 481 | A | A | A | A | A | A | T | N | | N | A | N |
| 482 | A | C | C | C | C | C | T | Y | | Y | A | N |
| 483 | T | T | T | T | T | T | T | T | | T | A | A/T |
| 484 | A | A | A | A | A | A | C | N | | N | G | N |
| 485 | T | T | T | T | T | T | T | T | | T | A | A/T |
| 486 | C | C | C | C | C | C | G | N | | N | A | N |
| 487 | G | G | G | G | G | G | A | R | | R | A | R |
| 488 | T | T | T | T | T | T | T | T | | T | A | T/A |
| 489 | T | T | T | T | T | T | G | N | | N | A | N |
| 490 | A | A | A | A | A | A | T | N | | N | A | N |
| 491 | T | T | T | T | T | T | A | N | | N | A | R |
| 492 | G | G | G | G | G | G | A | R | | R | A | N |
| 493 | T | A | T | A | T | N | A | N | | N | A | N |
| 494 | A | A | A | A | A | A | A | A | | A | A | A |
| 495 | A | A | A | A | A | A | A | A | | A | A | A |
| 496 | A | A | A | A | A | A | A | A | | A | A | A |
| 497 | A | A | A | A | A | A | A | A | | A | A | A |
| 498 | A | A | A | A | A | A | A | A | | A | A | A |
| 499 | A | A | A | A | A | A | A | A | | A | A | A |
| 500 | A | A | A | A | A | A | A | A | | A | A | A |
| 501 | A | A | A | A | A | A | A | A | | A | A | A |
| 502 | A | A | A | A | A | A | A | A | | A | A | A |
| 503 | A | A | A | A | A | A | A | A | | A | | A |
| 504 | A | A | A | A | A | A | A | A | | A | | A |
| 505 | A | A | A | A | A | A | A | A | | A | | A |
| 506 | A | A | A | A | A | A | A | A | | A | | A |
| 507 | A | A | A | A | A | A | A | A | | A | | A |
| 508 | A | A | A | A | A | A | A | A | | A | | A |
| 509 | A | A | A | A | A | A | A | A | | A | | A |
| 510 | A | A | A | A | A | A | A | A | | A | | A |
| 511 | A | A | A | A | A | A | A | A | | A | | A |
| 512 | | | | | | | | | | | | |

**FIG. 7.2 Cont.**

EP 2 279 760 A1 09-02-2011

| Position | Tm 12.84-2.2 | Tm 12.84-2.3 | Tm 12.84-3.4 | Tm 12.84-3.9 | Tm 12.84-7.5 | Consensus Tm 12.84 | Tm 13.17 | Consensus to Tm13.17 | Tm P-B1 | Tm P-B2 | Consensus to B1/B2 | Tm AFP-3 | Consensus to AFP-3 | GENERAL CONCENSUS | SUBSTITUTIONS - most to least common |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | M | M | M | M | M | M | M | | | M, OR GAP | M | M, OR GAP | M | |
| 2 | K | K | K | K | K | K | K | K | | | K, OR GAP | K | K, OR GAP | K | |
| 3 | L | L | L | L | L | L | L | L | | | L, OR GAP | L | L, OR GAP | L | |
| 4 | L | L | L | L | L | L | L | L | | | L, OR GAP | L | L, OR GAP | L | |
| 5 | L | L | L | L | L | L | C | HY-, ALP/SUL | | | HY-, ALP/SUL, OR GAP | L | HY-, ALP/SUL, OR GAP | L | C |
| 6 | C | C | C | C | C | C | C | C | | | C OR GAP | C | C OR GAP | C | |
| 7 | F | F | F | F | F | F | L | HY-, ARO/ALP | L | L | HY-, ARO/ALP | L | HY-, ARO/ALP | F | L |
| 8 | A | A | A | F | A | A | I | ALP | T | T | ALP/ALH | V | ALP/ALH | A | I T V |
| 9 | F | A | F | F | F | A | S | ARO/ALH | S | S | ARO/ALH | L | ARO/ALH/ALP | F | S L |
| 10 | A | A | A | A | A | A | L | ALP | L | L | ALP | V | ALP | A | L V |
| 11 | A | A | A | A | A | A | I | ALP | I | I | ALP | A | ALP | A | I |
| 12 | I | I | I | I | I | I | L | ALP | L | L | ALP | L | ALP | I | L |
| 13 | V | I | V | I | V | V | L | ALP | L | L | ALP | V | ALP | V | L |
| 14 | I | I | I | I | I | I | V | ALP | V | V | ALP | A | ALP | I | V A |
| 15 | G | G | G | G | G | G | T | ALP/ALH | A | A | ALP/ALH | A | ALP/ALH | G | T A T |
| 16 | A | A | A | A | A | A | V | ALP | V | V | ALP | T | ALP/ALH | A | V T |
| 17 | Q | Q | Q | Q | Q | Q | Q | Q | Q | Q | Q | Y | ACD/ARO | Q | Y |
| 18 | A | A | A | A | A | A | A | A | A | A | A | A | A | A | |
| 19 | L | L | L | L | L | L | T | L | I | I | ALP | | ALP OR GAP | L | I |
| 20 | T | T | T | T | T | T | T | T | | | T | | T OR GAP | T | |
| 21 | D | D | D | D | D | D | E | ACD | E | E | ACD | E | ACD | D | E |
| 22 | E | E | E | E | E | E | A | ACD/ALP | E | E | ACD/ALP | T | ACD/ALP/ALH | E | A T |
| 23 | Q | Q | Q | Q | Q | Q | Q | Q | D | D | ACD | P | ACD/ALP | Q | D P |
| 24 | I | I | Q | I | I | Q | I | I | L | L | ALP | R | ALP/BAS | I | L R |
| 25 | Q | Q | Q | Q | Q | Q | E | GLX | E | Q | GLX | E | GLX | Q | E L |
| 26 | K | K | K | K | K | K | K | K | L | L | BAS/ALP | K | BAS/ALP | K | L |
| 27 | R | R | R | R | R | R | L | BAS/ALP | L | L | BAS/ALP | L | BAS/ALP | R | L |
| 28 | N | N | N | K | N | K | N | N | R | R | HY+, BAS/ACD | K | HY+, BAS/ACD | N | R K |
| 29 | K | K | K | K | K | K | K | K | Q | Q | HY+, BAS/ACD | Q | HY+, BAS/ACD | K | Q |
| 30 | I | I | S | K | I | S | I | I | T | T | ALP/ALH | H | ALP/ALH/BAS | I | T H |
| 31 | S | S | K | S | K | K | S | S | S | S | S | S | S | S | |
| 32 | K | K | K | K | K | K | K | K | A | A | BAS/ALP | D | BAS/ALP/ACD | K | A D |
| 33 | E | E | E | C | E | C | K | HY+, ACD/BAS | E | E | HY+, ACD/BAS | A | ACD/BAS/ALP | E | A K |
| 34 | C | C | C | C | C | C | C | C | C | C | C | G | C | C | |
| 35 | Q | Q | Q | Q | Q | Q | Q | Q | K | K | HY+, ACD/BAS | K | HY+, ACD/BAS | Q | K |
| 36 | Q | Q | Q | Q | Q | Q | N | ACD | T | T | HY+, ACD/ALH | A | ACD/ALH/ALP | Q | N T A |
| 37 | V | V | V | V | E | V | E | ACD/ALP | E | E | ACD/ALP | E | ACD/ALP | E | V (3.9, Tm13.17, B1, B2, AFP-3) |
| 38 | S | S | S | S | S | S | S | S | S | S | S | S | S | S | |
| 39 | G | G | G | G | G | G | G | G | G | G | G | G | G | G | |
| 40 | V | V | V | V | V | S | G | V | V | A | ALP | V | ALP | V | A |
| 41 | S | S | S | S | S | S | V | S | S | S | S | S | S | S | |
| 42 | Q | Q | Q | Q | Q | Q | S | Q | E | E | GLX | E | GLX | Q | E |
| 43 | E | T | E | T | E | T | Q | E | D | A | ACD/ALP | E | ACD/ALP | E | D A |
| 44 | T | T | T | T | T | T | E | I | V | V | ALP | S | ALP/ALH | T | I V S |
| 45 | I | I | I | I | I | I | I | I | I | I | I | L | ALP | L | L |
| 46 | D | D | D | D | D | D | I | HY+, ACD/ALH | K | K | HY+, ACD/ALH/BAS | N | HY+, ACD/ALH/BAS | D | T K N |
| 47 | K | K | K | K | K | K | K | K | R | R | BAS | K | BAS | K | R |
| 48 | V | V | V | V | V | V | A | ALP | A | A | ALP | V | ALP | V | A |
| 49 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | |
| 50 | T | T | T | G | T | T | N | HY+, ALH/ACD | K | K | HY+, ALH/ACD/BAS | N | HY+, ALH/ACD/BAS | T | N K |
| 51 | G | V | G | V | G | V | G | G | G | G | G | R | ALP/BAS | G | R |
| 52 | V | V | V | V | V | V | D | ALP/ACD | D | D | ALP/ACD | E | ALP/ACD | V | D E |
| 53 | L | L | L | V | L | L | W | HY-, ALP/ARO | L | L | HY-, ALP/ARO | E | ALP/ARO/ACD | L | W E |
| 54 | V | V | V | V | V | V | E | ALP/ACD | E | E | ALP/ACD | V | ALP/ACD | V | E |
| 55 | D | D | D | D | D | D | D | D | D | D | D | D | D | D | |
| 56 | D | D | D | D | D | D | D | D | D | D | D | D | D | D | |
| 57 | P | P | P | P | P | P | P | P | P | P | P | P | P | P | |
| 58 | K | K | K | K | K | K | K | K | K | K | K | K | K | K | |
| 59 | M | M | M | M | M | M | L | HY-, SUL/ALP | L | L | HY-, SUL/ALP | L | HY-, SUL/ALP | M | L |
| 60 | K | K | K | K | K | K | K | K | K | K | K | K | K | K | |
| 61 | K | K | K | K | K | K | R | BAS | M | Q | BAS/SUL | E | BAS/SUL/ACD | K | R M E |
| 62 | H | H | H | H | H | H | Q | HY+, BAS/ACD | Q | Q | HY+, BAS/ACD | H | HY+, BAS/ACD | H | Q |
| 63 | V | V | V | V | V | V | V | V | L | L | ALP | A | ALP | V | L A |
| 64 | L | L | L | L | L | L | F | HY-, ALP/ARO | L | L | HY-, ALP/ARO | F | HY-, ALP/ARO | L | F |
| 65 | C | C | C | C | C | C | C | C | C | C | C | C | C | C | |
| 66 | F | F | F | F | F | F | F | HY-, ARO/ALP | I | I | HY-, ARO/ALP | L | HY-, ARO/ALP | F | V I |
| 67 | S | S | S | S | S | S | A | ALH/ALP | F | F | ALH/ALP/ARO | L | ALH/ALP/ARO | S | A F L |
| 68 | K | K | K | K | K | K | R | BAS | K | K | BAS | K | BAS | K | R |
| 69 | K | K | K | K | R | K | N | HY+, BAS/ACD | A | A | BAS/ACD/ALP | R | BAS/ACD/ALP | R | A N (2.2, 2.3, 3.4, 7.5) |
| 70 | T | T | T | T | T | T | A | ALP | L | L | ALP | A | ALP | T | L A |
| 71 | G | V | G | V | G | G | G | G | I | E | ALP/ACD | G | ALP/ACD | G | E I |
| 72 | V | V | A | V | A | V | L | ALP | E | I | ALP/ACD | F | ALP/ACD/ARO | V | L E F |
| 73 | A | A | A | A | A | A | A | A | V | V | ALP | I | ALP | A | V I |
| 74 | T | T | T | T | T | T | T | T | A | A | ALH/ALP | D | ALH/ALP/ACD | T | A D |
| 75 | E | E | E | E | E | E | E | E | E | S | ACD/ALP | E | ACD/ALP | A | E A |
| 76 | A | A | A | A | A | A | S | ALP/ALH | S | S | ALP/ALH | S | ALP/ALH | S | A |
| 77 | G | G | G | G | G | G | G | G | G | G | G | G | G | G | |
| 78 | D | D | D | D | D | D | E | ACD | E | E | ACD | E | ACD | E | D |
| 79 | T | T | N | V | T | N | V | ALP | I | I | ALP | F | ALP/ARO | T | V F |
| 80 | N | N | N | V | N | N | V | ACD/ALP | E | E | ACD/ALP | Q | ACD/ALP | N | V Q E |
| 81 | V | V | V | V | V | V | V | V | A | A | ALP | L | ALP | V | L A |
| 82 | E | E | V | E | V | E | D | ACD | D | D | ACD | D | ACD | D | E |
| 83 | V | V | V | V | V | V | V | V | T | T | ALP/ALH | H | ALP/ALH/BAS | V | T H |
| 84 | L | L | L | L | L | L | L | L | F | F | ALP/ARO | I | ALP/ARO | L | I |
| 85 | K | K | K | K | K | K | R | BAS | K | K | BAS | K | BAS | K | R |
| 86 | A | A | A | A | A | A | E | ALP/ACD | E | E | ALP/ACD | T | ALP/ACD/ALH | A | E T |
| 87 | K | K | K | K | K | K | K | K | K | K | K | K | K | K | |
| 88 | L | L | L | K | L | L | V | ALP | L | L | ALP | F | ALP/ARO | L | V F |
| 89 | K | K | K | K | K | K | R | BAS | T | T | BAS/ALH | K | BAS/ALH | K | R |
| 90 | H | H | H | H | H | H | K | BAS | R | R | BAS | E | BAS/ACD | H | R K E |
| 91 | V | V | V | V | V | V | V | V | V | V | V | N | ALP/ACD | V | |
| 92 | A | A | A | A | A | A | T | ALP/ALH | T | T | ALP/ALH | S | ALP/ALH | A | S |
| 93 | S | S | S | S | S | S | D | HY+, ALH/ACD | N | N | HY+, ALH/ACD | E | HY+, ALH/ACD | S | N D E |
| 94 | | | | | | | N | N OR GAP | D | D | ACD OR GAP | H | ACD OR GAP | D | H |
| 95 | D | D | D | D | D | D | D | D | D | D | D | P | ACD/ALP | D | P |
| 96 | E | E | E | E | E | E | E | E | E | E | E | E | E | E | |
| 97 | E | E | E | E | E | E | E | E | E | E | E | K | HY+, ACD/BAS | E | K |

FIG. 7.3

TD2090 "9+E9Z860

| Position | Tm 12.84-2.2 | Tm 12.84-2.3 | Tm 12.84-3.4 | Tm 12.84-3.9 | Tm 12.84-7.5 | Concensus | Tm 13.17 | Concensus | Tm P-B1 | Tm P-B2 | Concensus | Tm AFP-3 | Concensus | GENERAL | SUBSTITUTIONS - most to least common | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 98 | V | V | V | V | V | V | T | ALP/ALH | S | S | ALP/ALH | V | ALP/ALH | V | S | T | |
| 99 | D | D | D | D | D | D | E | ACD | E | E | ACD | D | ACD | D | E | | |
| 100 | K | K | K | K | K | K | K | K | K | K | K | D | HY+, BAS/ACD | K | D | | |
| 101 | I | I | I | I | I | I | I | I | I | I | I | L | ALP | I | L | | |
| 102 | V | V | V | V | V | V | I | ALP | V | V | ALP | V | ALP | V | I | | |
| 103 | Q | Q | Q | Q | Q | Q | N | ACD | E | E | ACD | A | ACD/ALP | Q | E | N | A |
| 104 | K | K | K | K | K | K | K | K | K | K | K | K | K | K | | | |
| 105 | C | C | C | C | C | C | C | C | C | C | C | C | C | C | | | |
| 106 | V | V | V | V | V | V | A | ALP | T | T | ALP/ALH | A | ALP/ALH | V | A | T | |
| 107 | V | V | V | V | V | V | V | V | V | V | V | V | V | V | | | |
| 108 | K | K | K | K | K | K | K | K | T | T | HY+, ACD/ALH | K | HY+, ACD/ALH | K | T | | |
| 109 | K | K | K | K | K | K | R | BAS | E | E | HY+, BAS/ACD | K | HY+, BAS/ACD | K | E | R | |
| 110 | A | A | A | A | A | A | D | ALP/ACD | D | D | ALP/ACD | D | ALP/ACD | D | A | | |
| 111 | T | T | T | T | T | T | T | T | T | T | T | T | T | T | | | |
| 112 | P | P | P | P | P | P | V | ALP | P | P | ALP | P | ALP | P | V | | |
| 113 | E | E | E | E | E | E | E | E | E | E | E | Q | ACD | E | Q | | |
| 114 | E | E | E | E | E | E | E | E | D | D | ACD | H | HY+, ACD/BAS | E | D | H | |
| 115 | T | T | T | T | T | T | T | T | T | T | T | S | ALH | T | S | | |
| 116 | A | A | A | A | A | A | V | ALP | A | A | ALP | S | ALP/ALH | A | V | S | |
| 117 | Y | Y | Y | Y | Y | Y | F | ARO | F | F | ARO | A | ARO/ALP | Y | F | A | |
| 118 | D | D | D | D | D | D | N | ASX | E | E | ACD | D | ACD | D | E | N | |
| 119 | T | T | T | T | T | T | T | T | V | V | ALH/ALP | F | ALH/ALP/ARO | T | V | F | |
| 120 | F | F | F | F | F | F | F | F | T | T | ARO/ALH | F | ARO/ALH/ARO | F | T | | |
| 121 | K | K | K | K | K | K | K | K | K | K | K | K | K | K | | | |
| 122 | C | C | V | C | C | C | C | HY-, SUL/ALP | C | C | HY-, SUL/ALP | C | HY-, SUL/ALP | C | V | (3.4 is the only variant in the family) | |
| 123 | I | I | I | I | I | I | V | ALP | V | V | ALP | V | ALP | V | I | | |
| 124 | Y | Y | Y | Y | Y | Y | M | ARO/SUL | L | L | ARO/SUL/ALP | H | ARO/SUL/ALP/BAS | Y | L | M | H |
| 125 | D | D | D | D | D | D | K | HY+, ACD/BAS | K | K | HY+, ACD/BAS | D | HY+, ACD/BAS | D | K | | |
| 126 | S | S | S | S | S | S | N | HY+, ALH/ACD | D | D | HY+, ALH/ACD | N | HY+, ALH/ACD | S | N | D | |
| 127 | K | K | K | K | K | K | K | K | K | K | K | R | BAS | K | R | | |
| 128 | P | P | P | P | P | P | P | P | P | P | P | S | ALP/ALH | P | S | | |
| 129 | D | D | D | D | D | D | K | HY+, ACD/BAS | N | N | HY+, ACD/BAS OR GAP | | HY+, ACD/BAS OR GAP | D | N | K | |
| 130 | F | F | F | F | F | F | F | F | F | F | F | | F OR GAP | F | | | |
| 131 | S | S | S | S | S | S | S | S | F | F | ALH/ARO | | ALH/ARO OR GAP | S | F | | |
| 132 | P | P | P | P | P | P | P | P | G | G | ALP | | ALP OR GAP | P | G | | |
| 133 | I | I | I | I | I | I | V | ALP | D | D | ALP/ACD | | ALP/ACD OR GAP | I | D | V | |
| 134 | D | D | D | D | D | D | D | D | L | L | ACD/ALP | | ACD/ALP OR GAP | D | L | | |
| 135 | | | | | | | | | F | F | F, OR GAP | | F, OR GAP | F | | | |
| 136 | | | | | | | | | V | V | V, OR GAP | | V, OR GAP | V | | | |
| 137 | | | | | | | | | | | | | | | | | |
| 138 | | | | | | | | | | | | | | | | | |

**FIG. 7.3 Cont.**

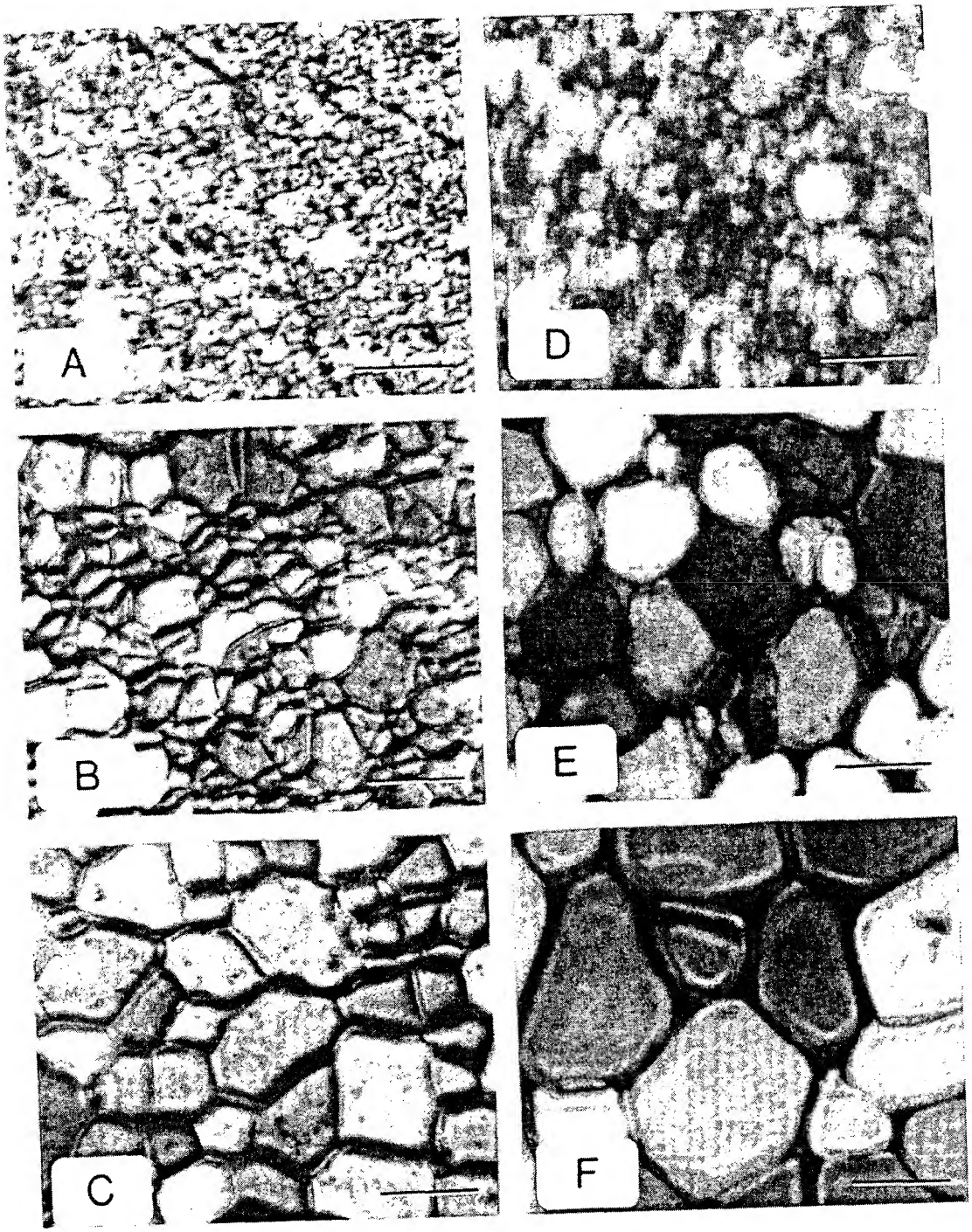



FIG. 8.0

EP 2 908 868 A1

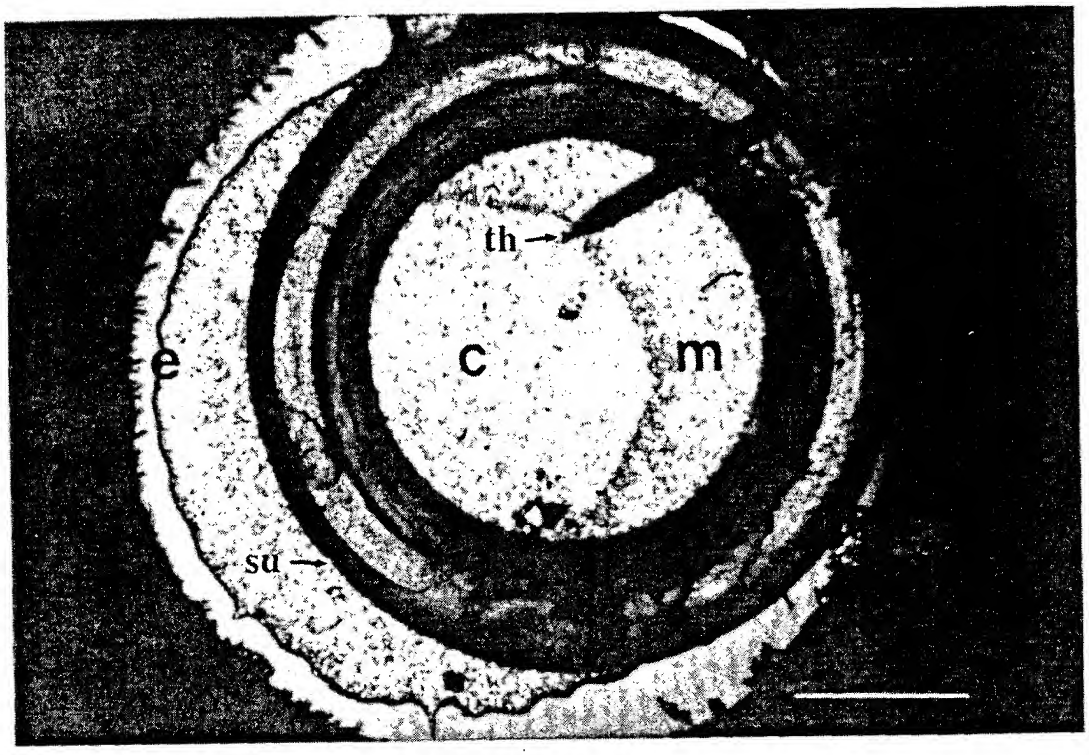



FIG. 8.1a

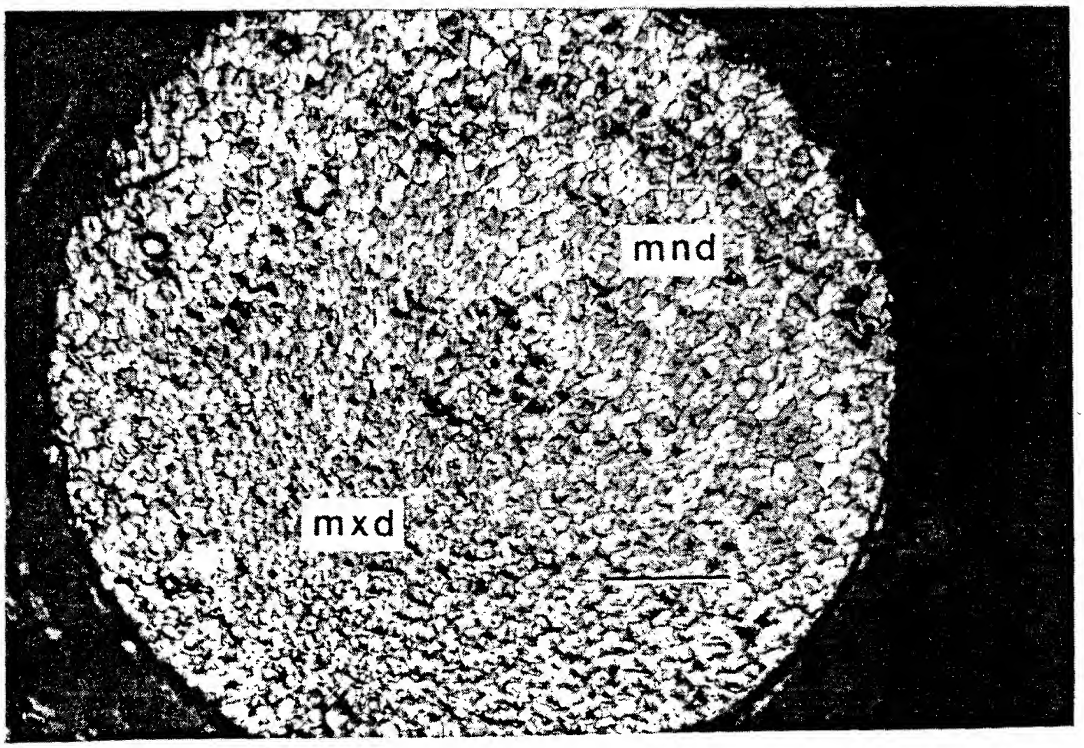
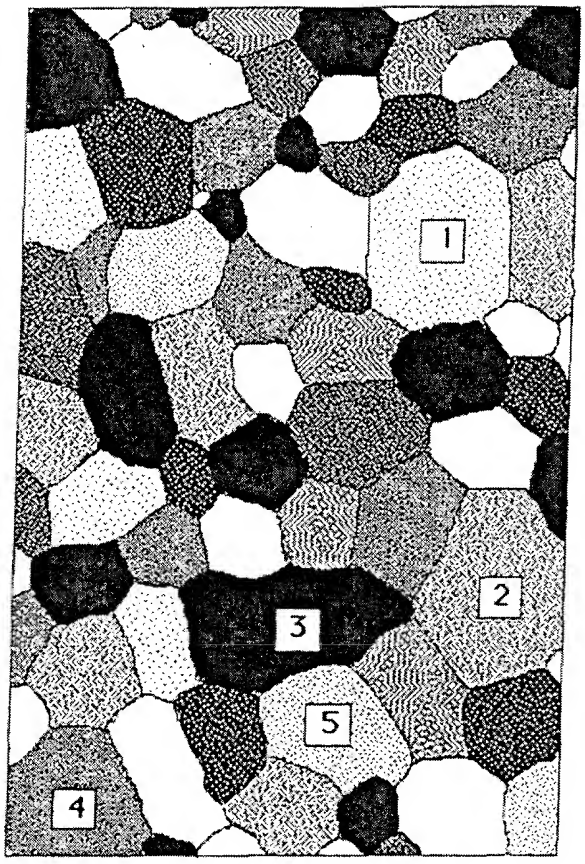
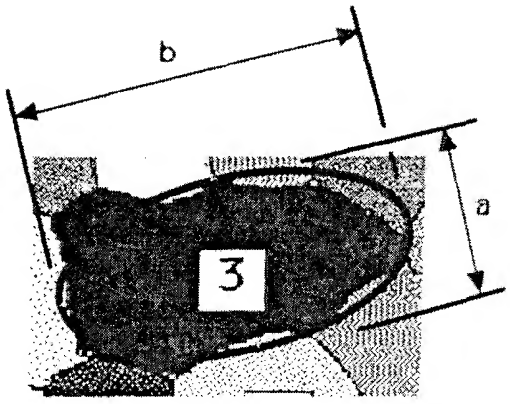



FIG. 8.1b




grain area=0.25πab

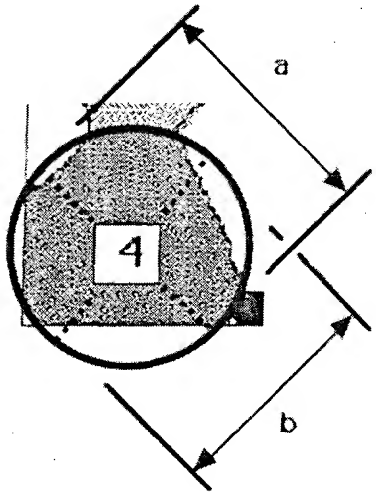



FIG. 8.2

EP 2 908 848 A2

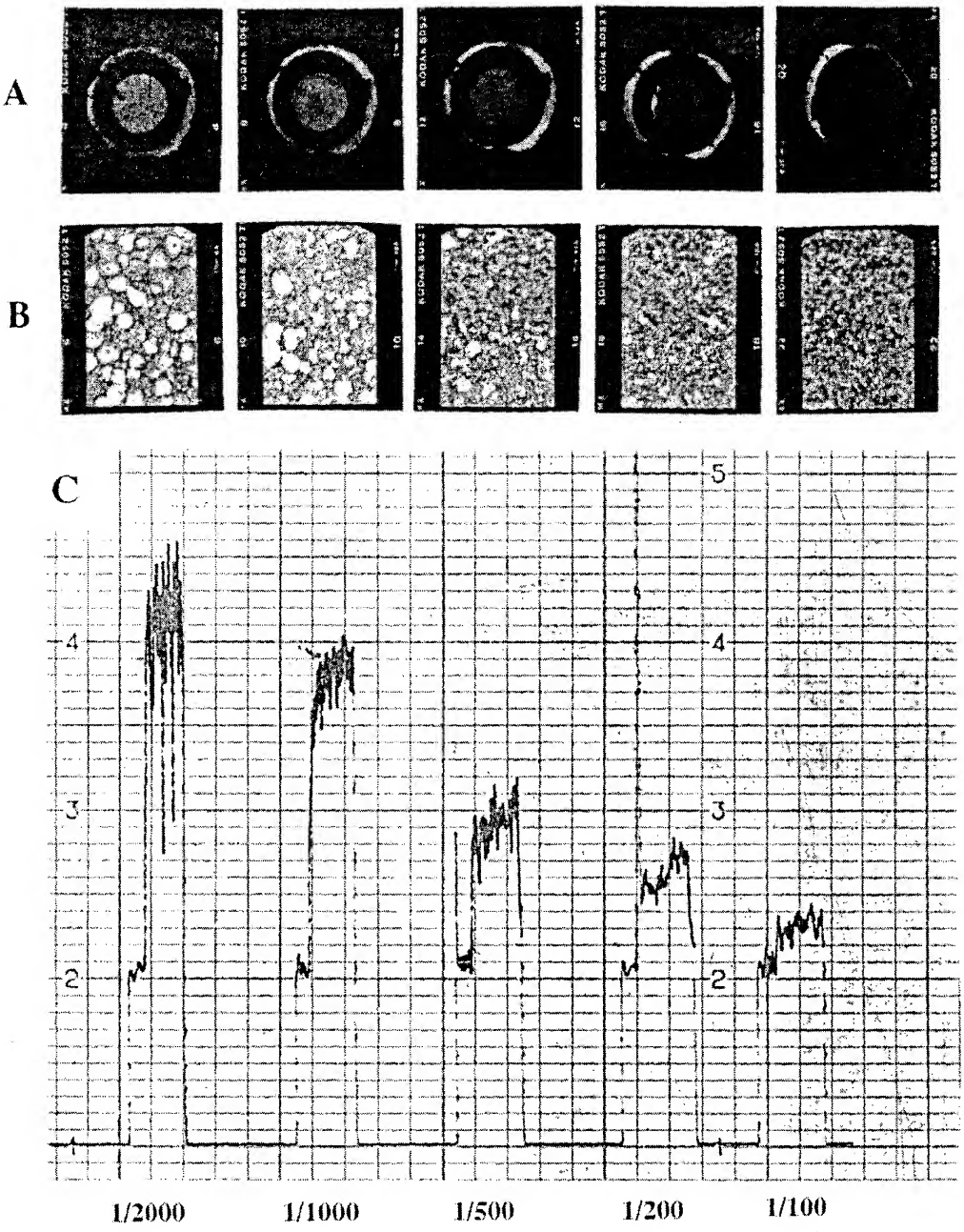



FIG. 8.3

FP6268-6+2-9-2850

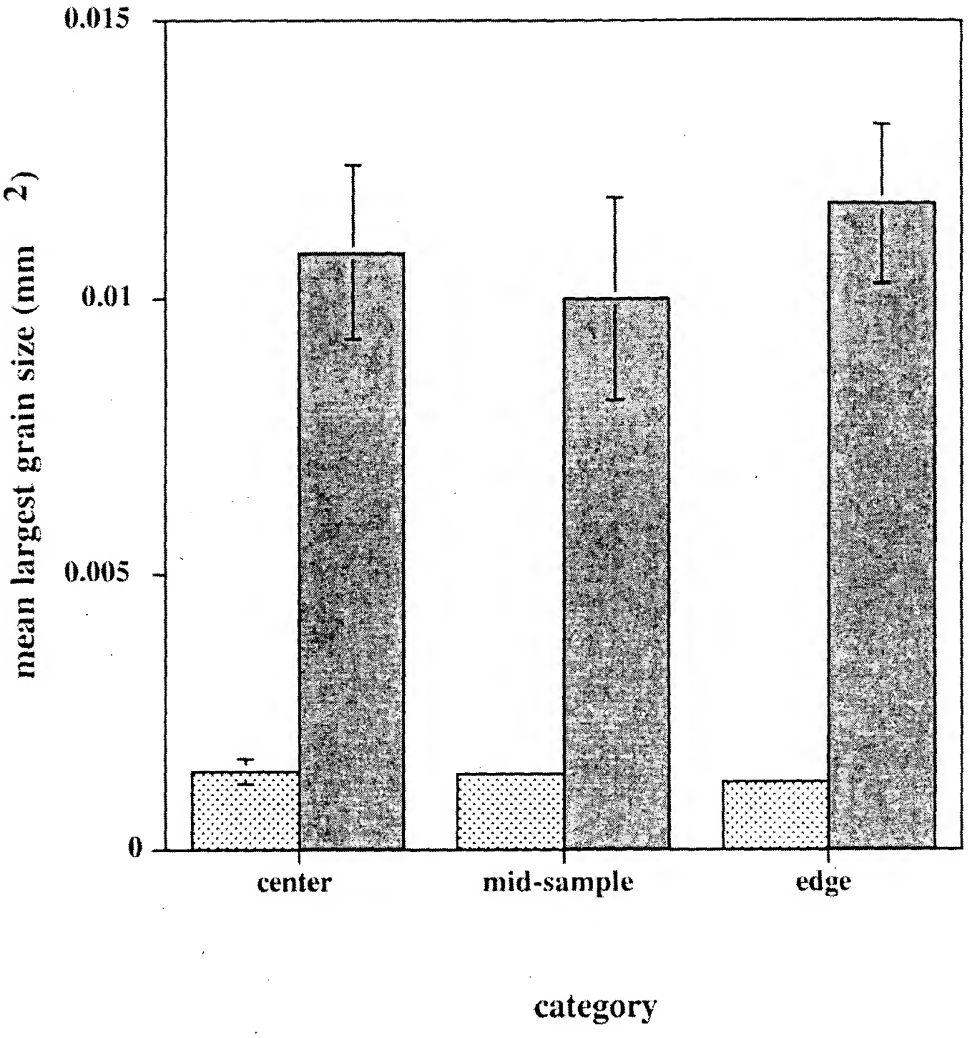



FIG. 8.4a

EP 2 756 868 A1

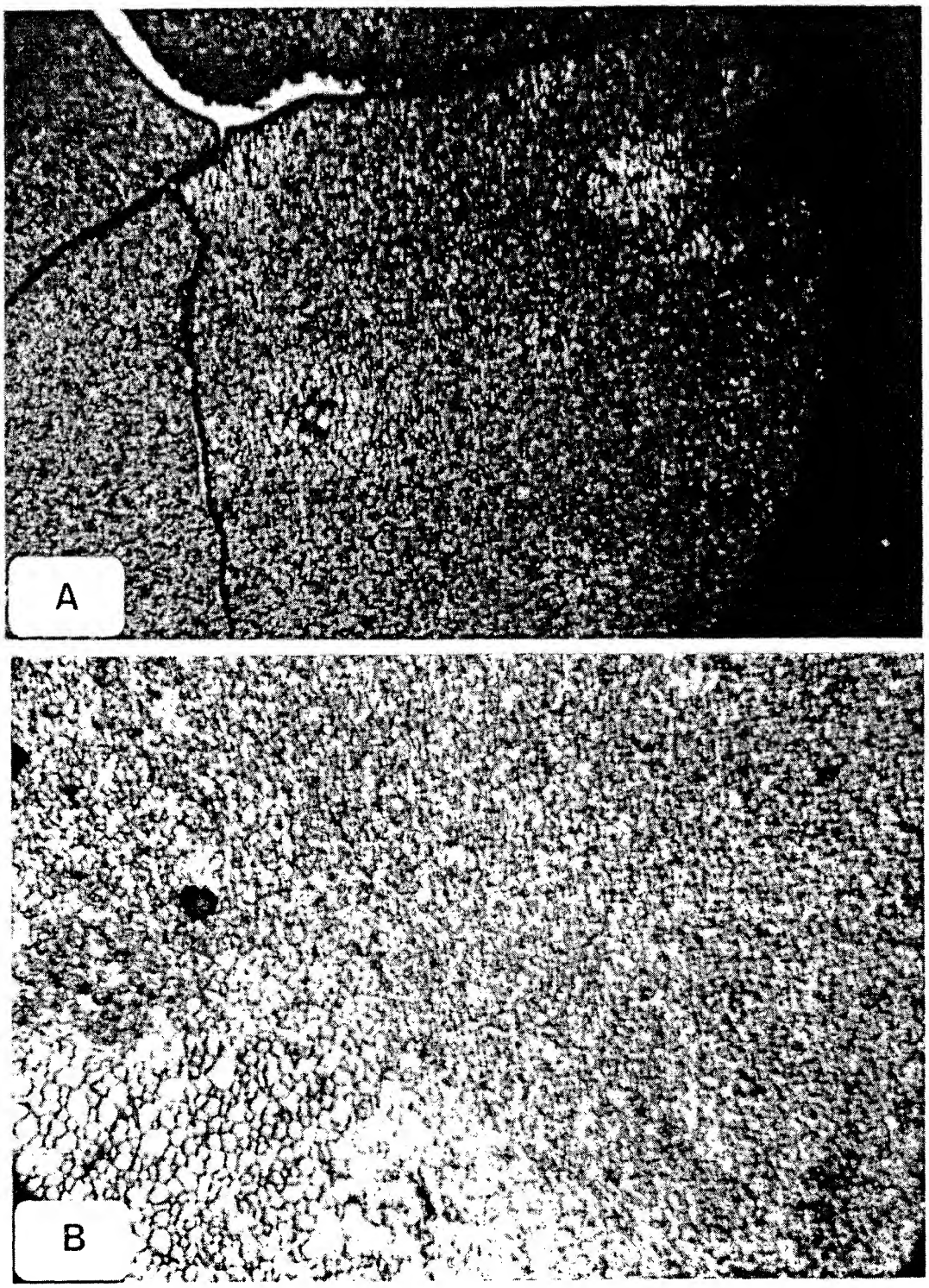



FIG. 8.4b

F0/090-6+E9/960

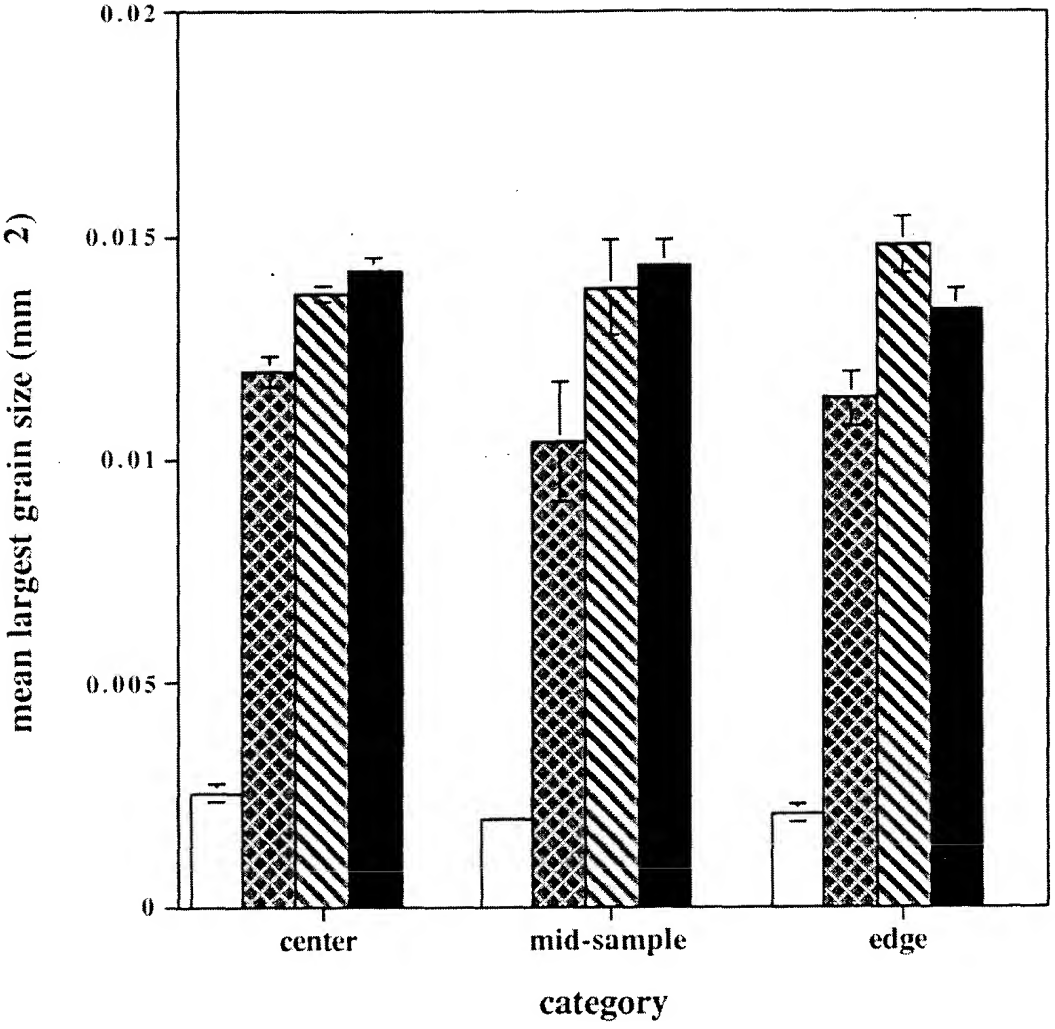



FIG. 8.5a

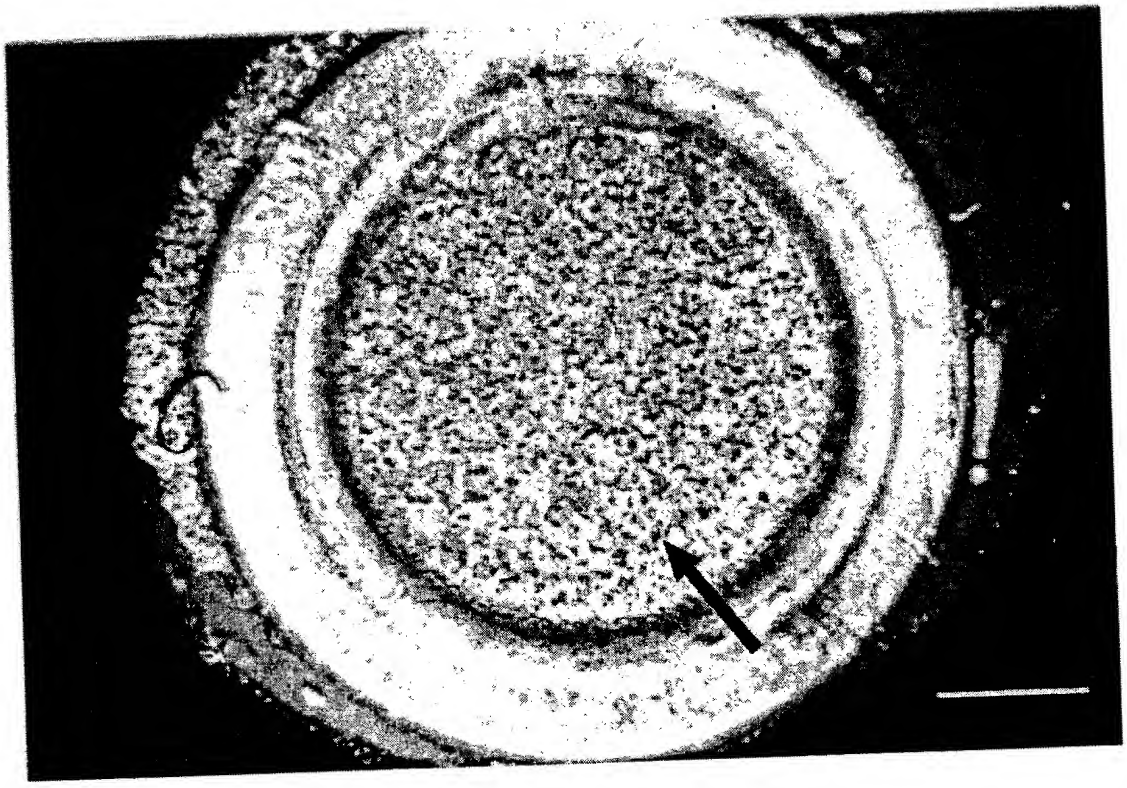


FIG. 8.5b

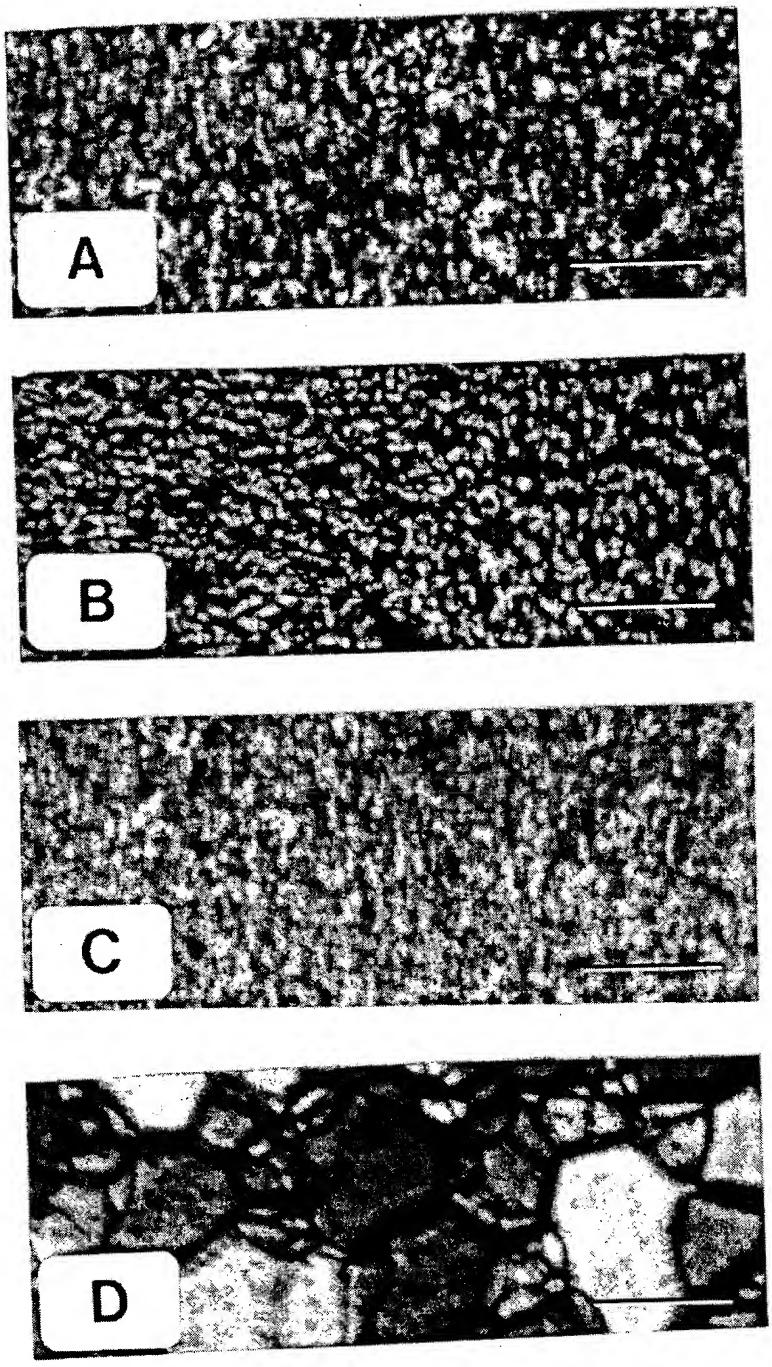



FIG. 8.6

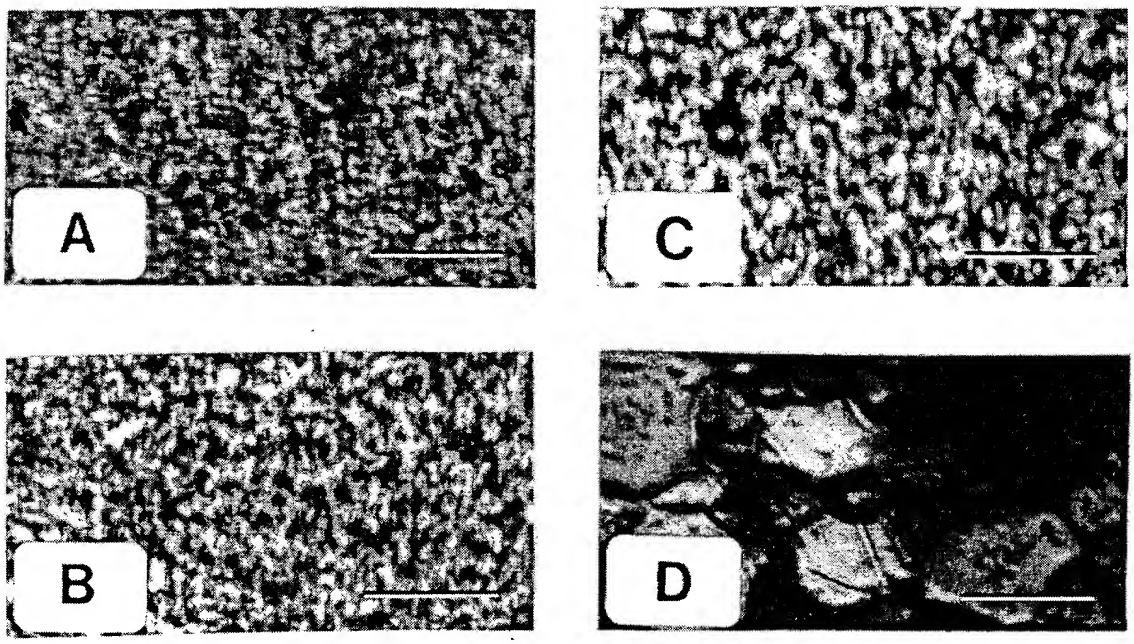



FIG. 8.7

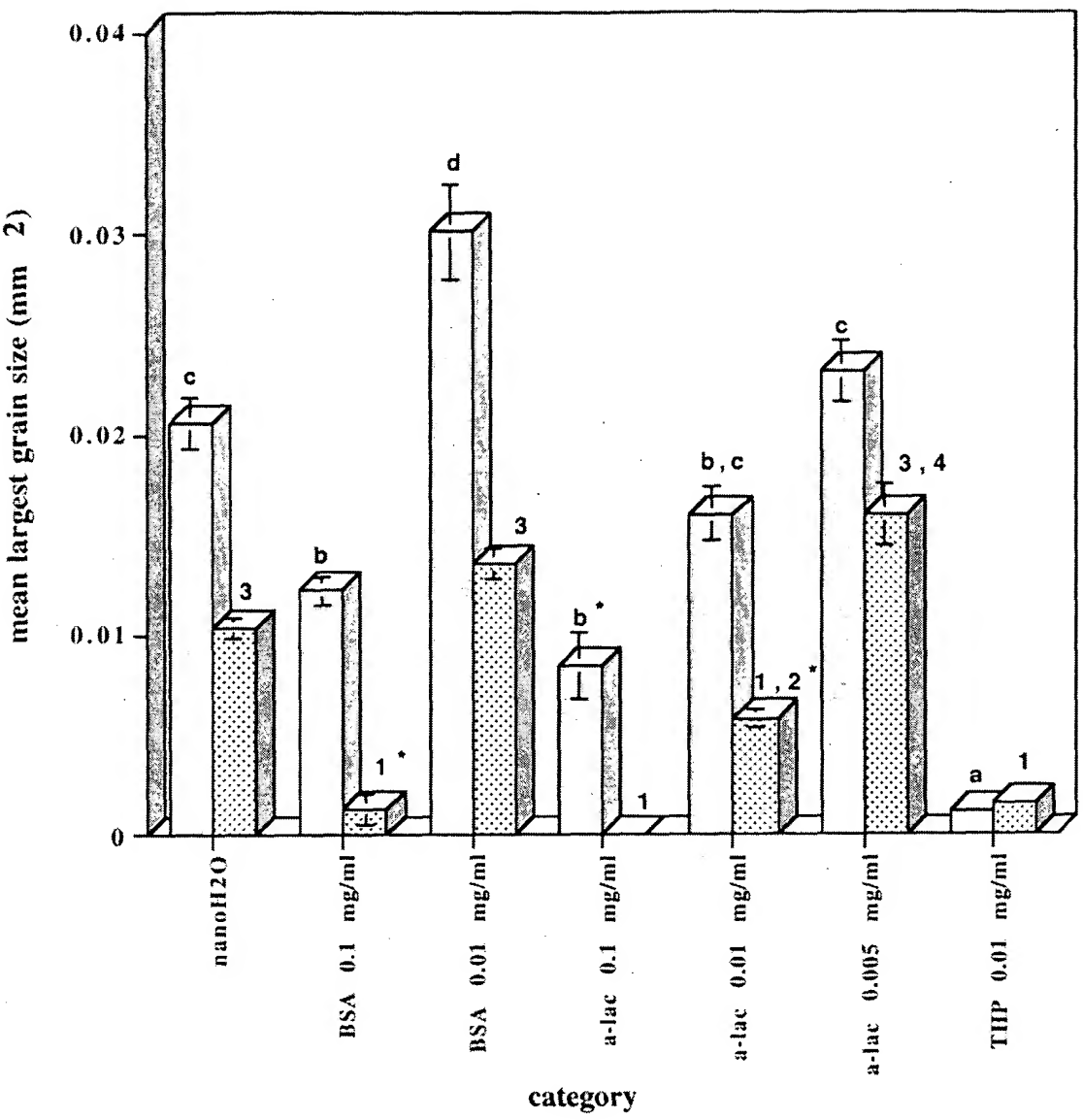

mean largest grain size (mm 2)
0.04
0.03
0.02
0.01
0
c
3
b
1 *
d
3
b *
1
b , c
1 , 2 *
c
3 , 4
a
1
nanoH2O
BSA 0.1 mg/ml
BSA 0.01 mg/ml
a-lac 0.1 mg/ml
a-lac 0.01 mg/ml
a-lac 0.005 mg/ml
THP 0.01 mg/ml
category

FIG. 8.8

TP2009-0342760

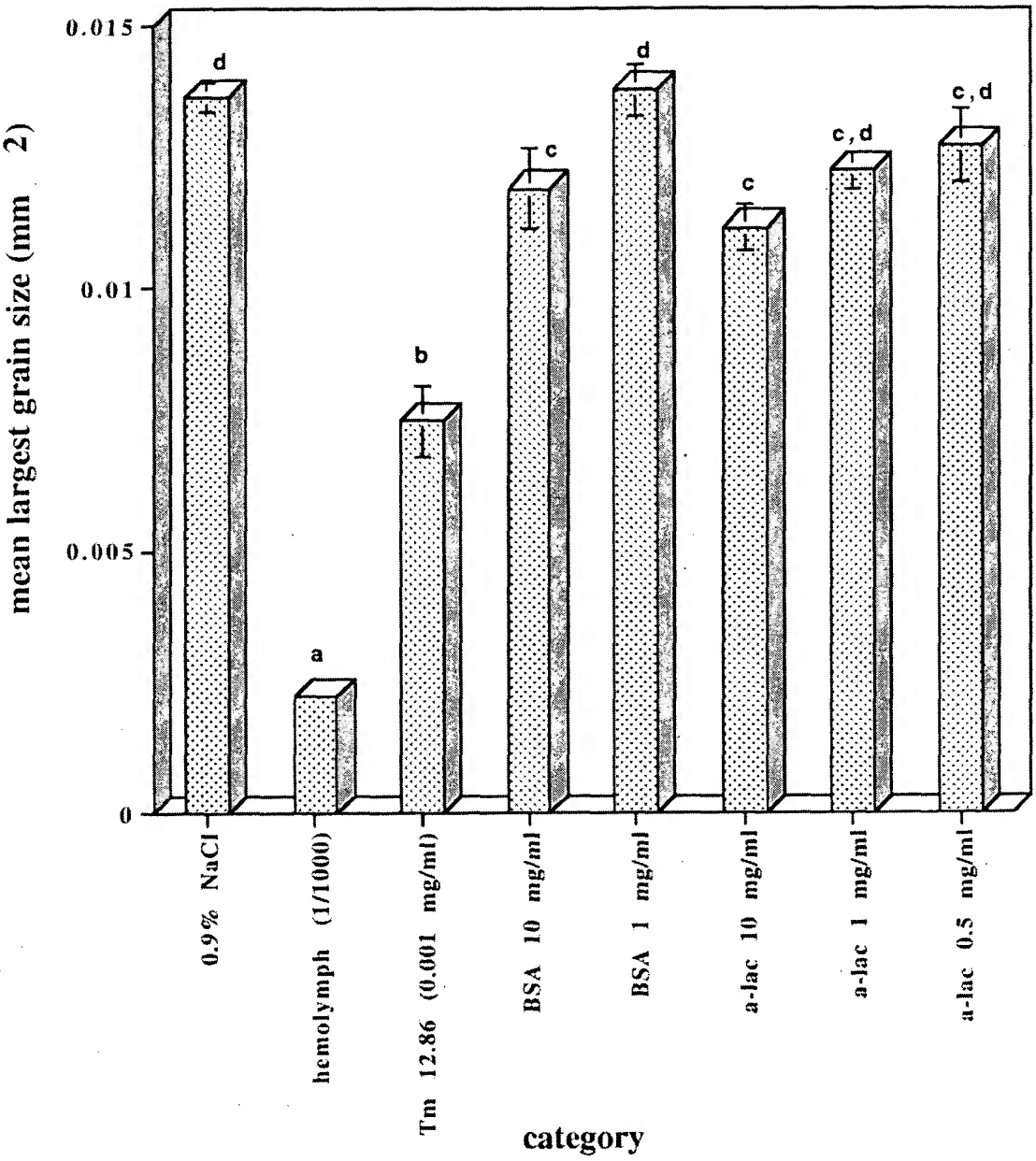



FIG. 8.9

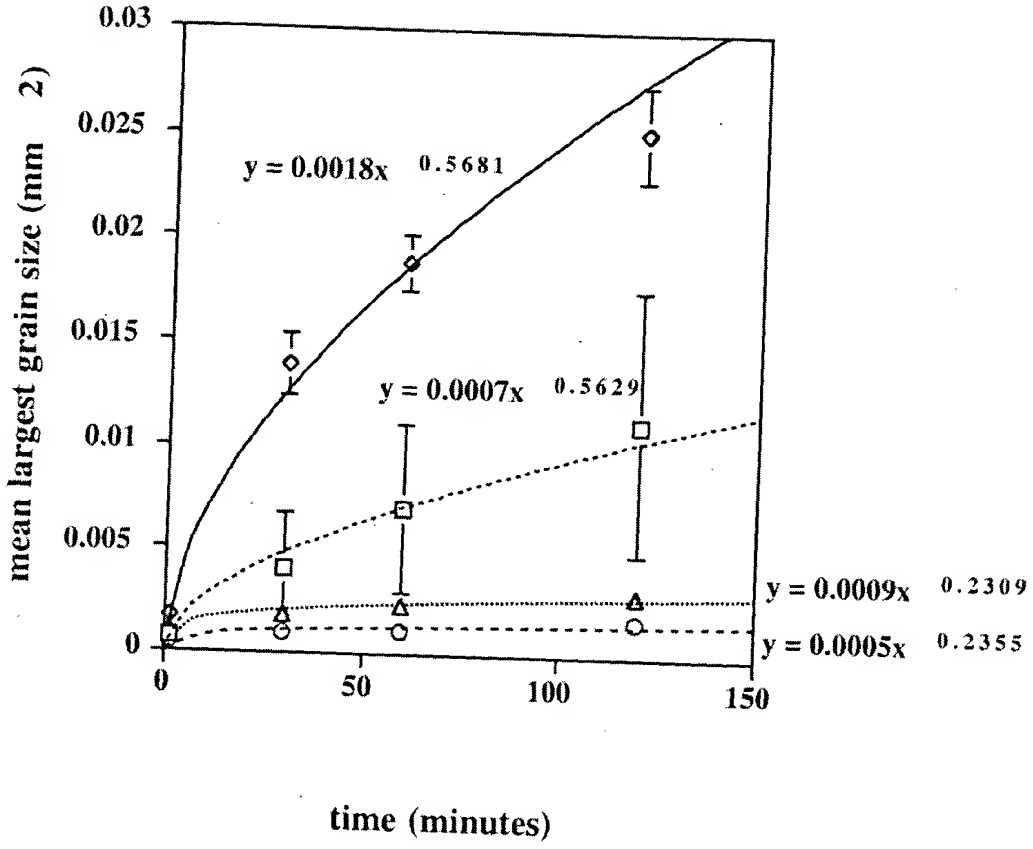

0.03
0.025
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
y = 0.0018x 0.5681
y = 0.0007x 0.5629
y = 0.0009x 0.2309
y = 0.0005x 0.2355
0
50
100
150
time (minutes)

FIG. 8.10

T0209P-EP2342860

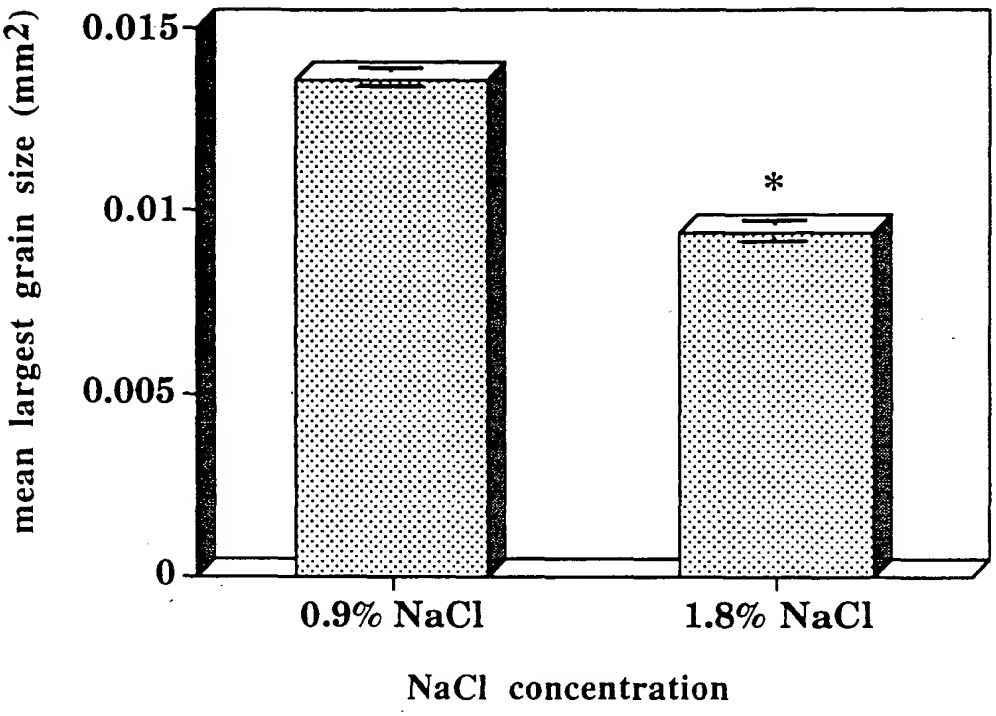



FIG. 8.11

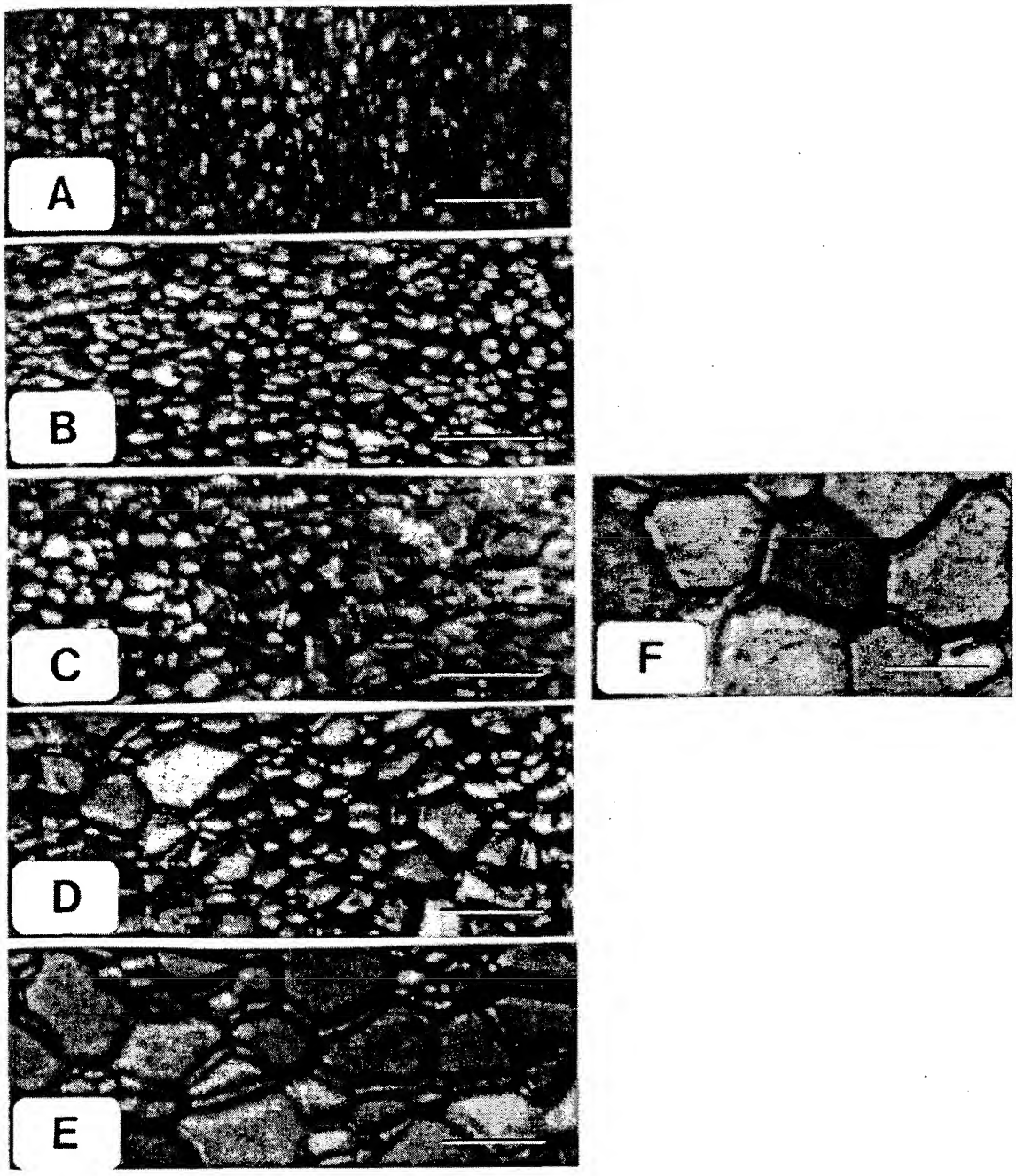



FIG. 8.12

EP 2 030 634 A1

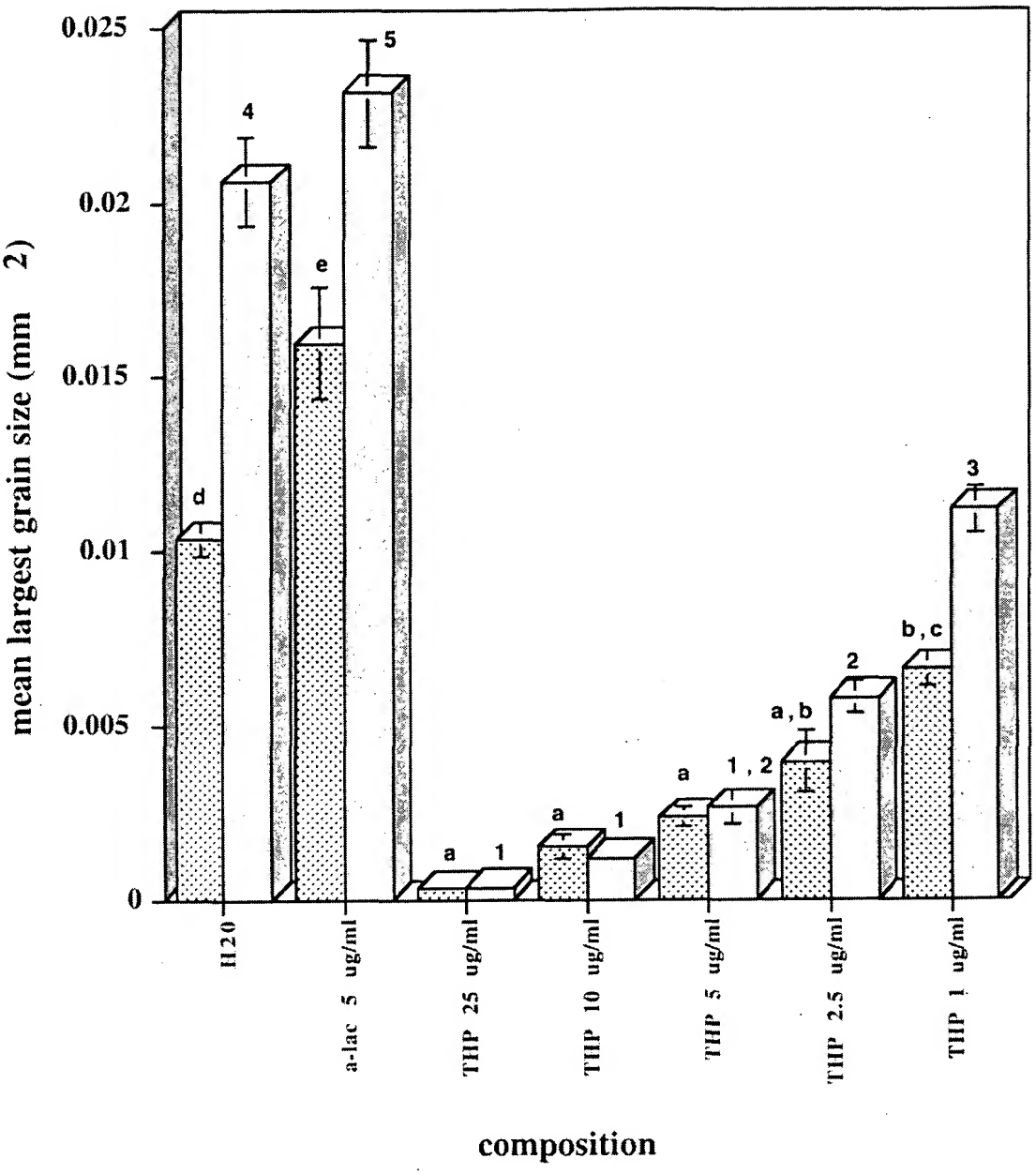



FIG. 8.13

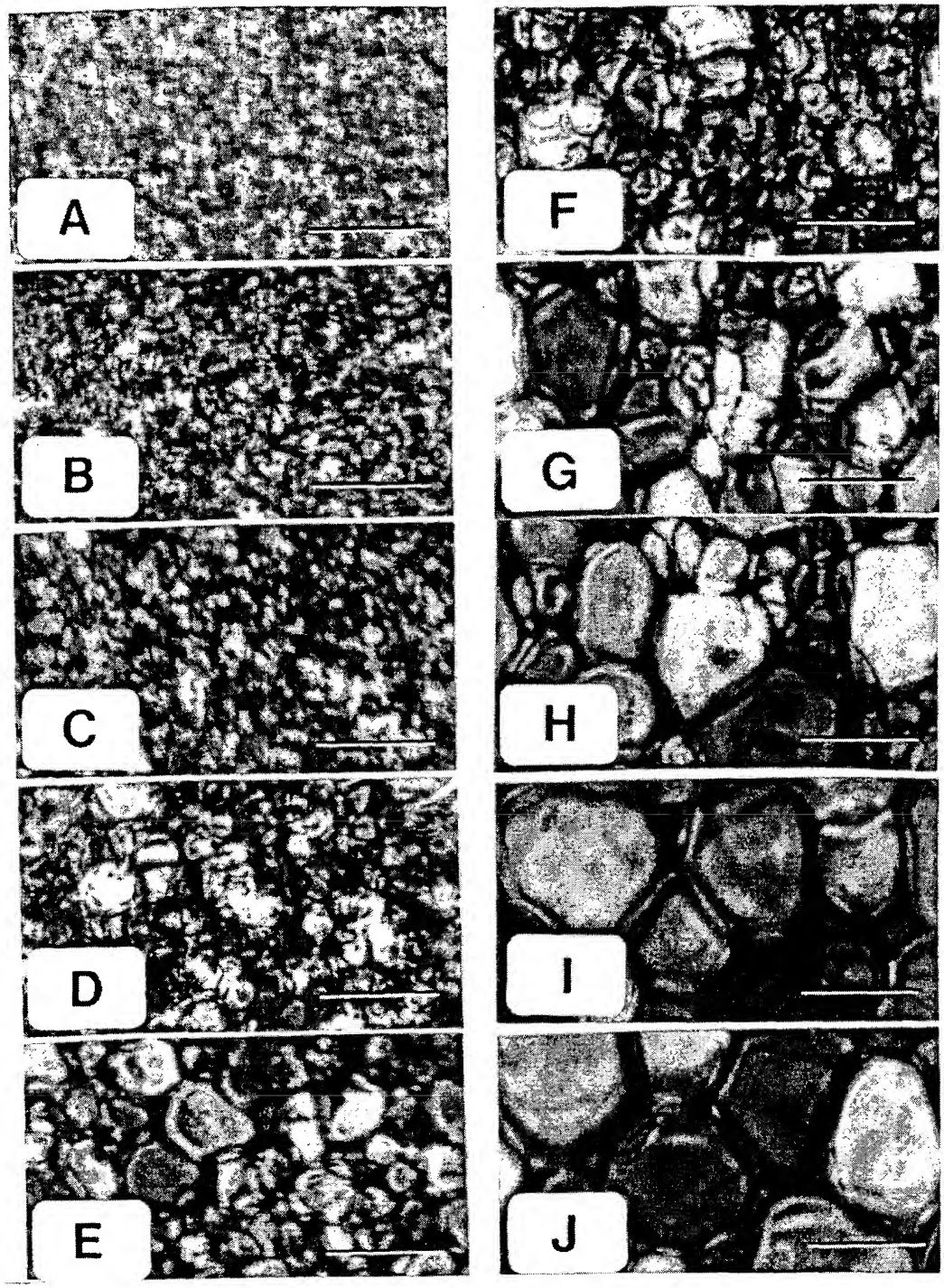



FIG. 8.14

TO2090 BHE92860

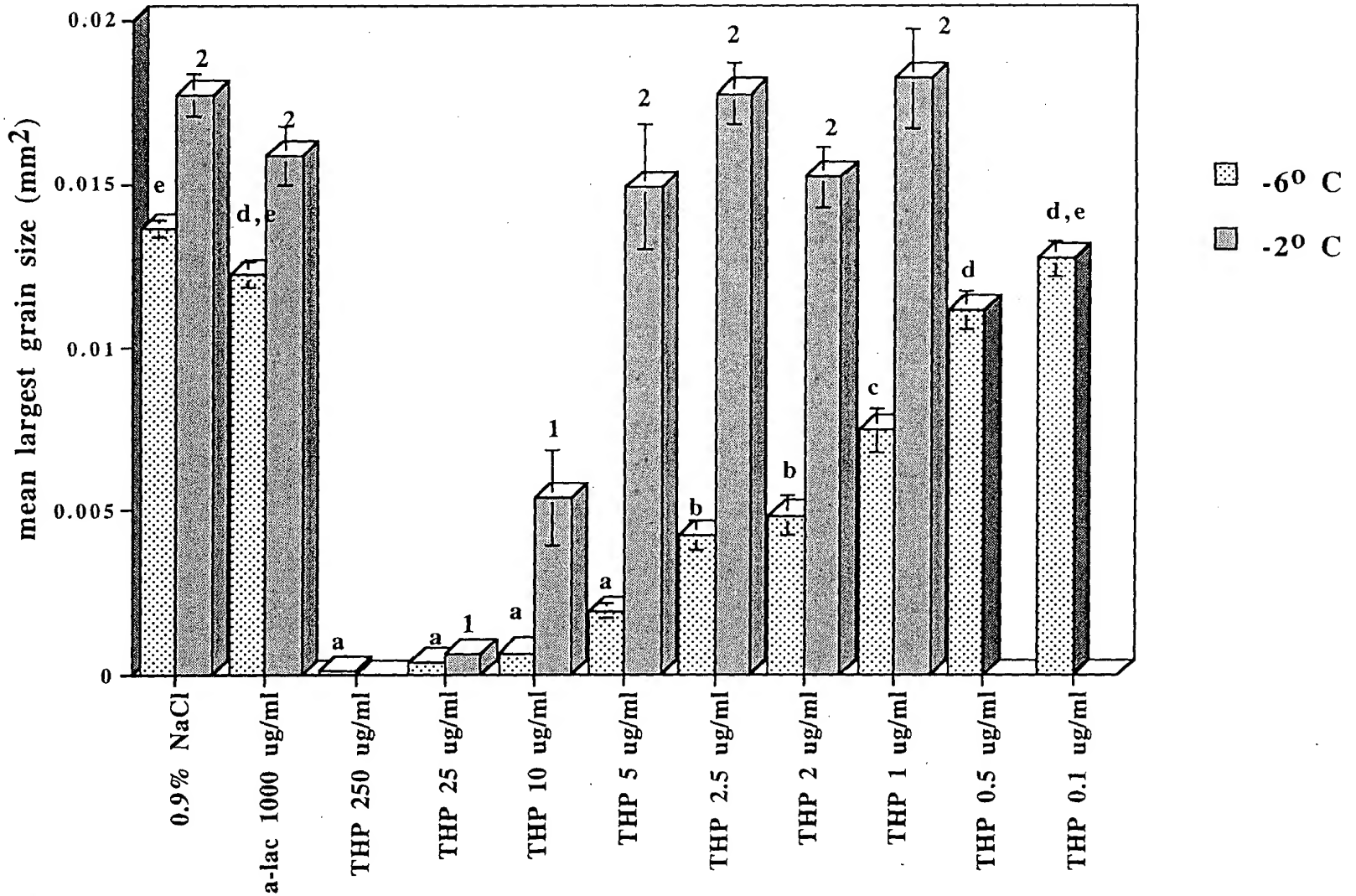



FIG. 8.15

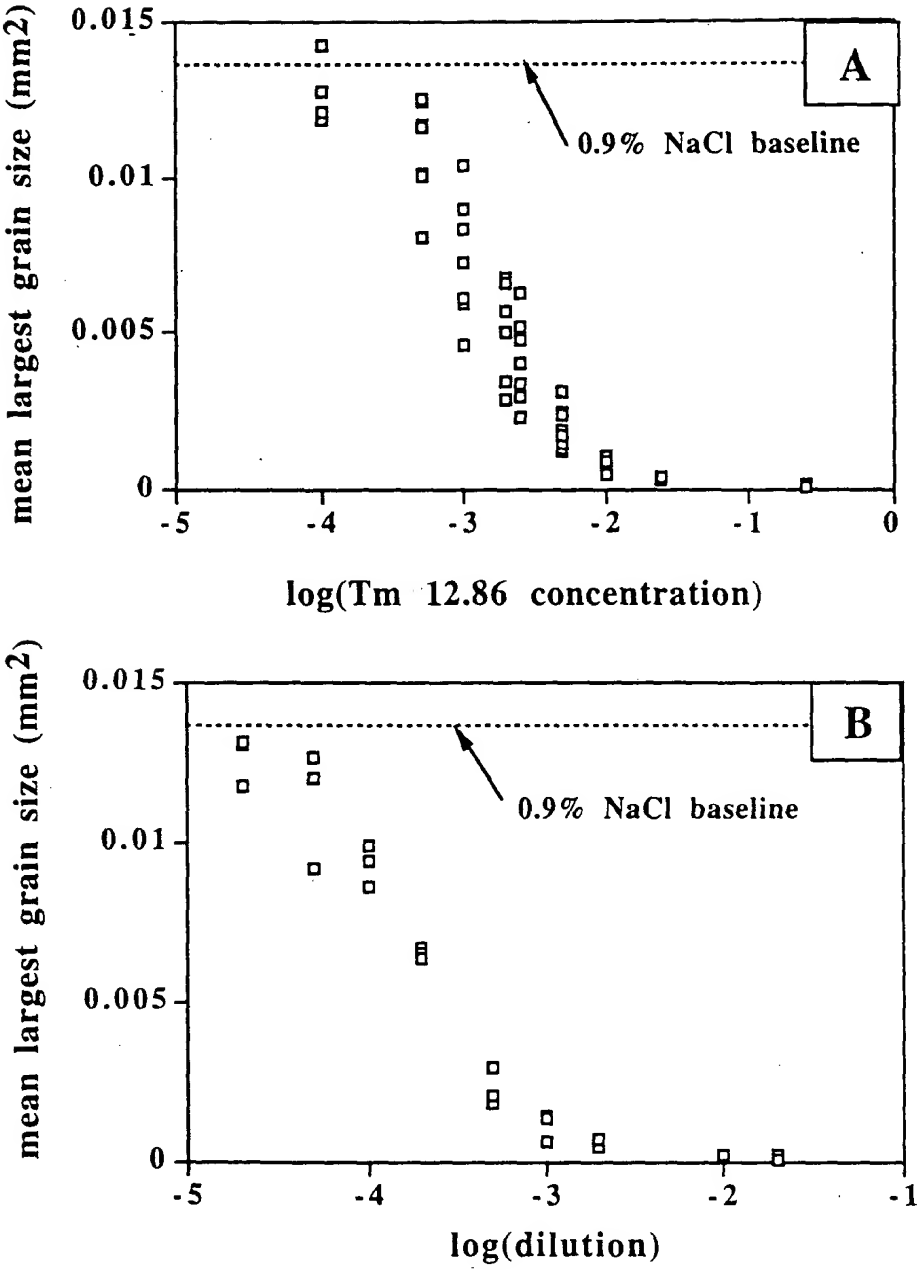

A
0.9% NaCl baseline
mean largest grain size (mm2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
0
log(Tm 12.86 concentration)
B
0.9% NaCl baseline
mean largest grain size (mm2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
log(dilution)

FIG. 8.16

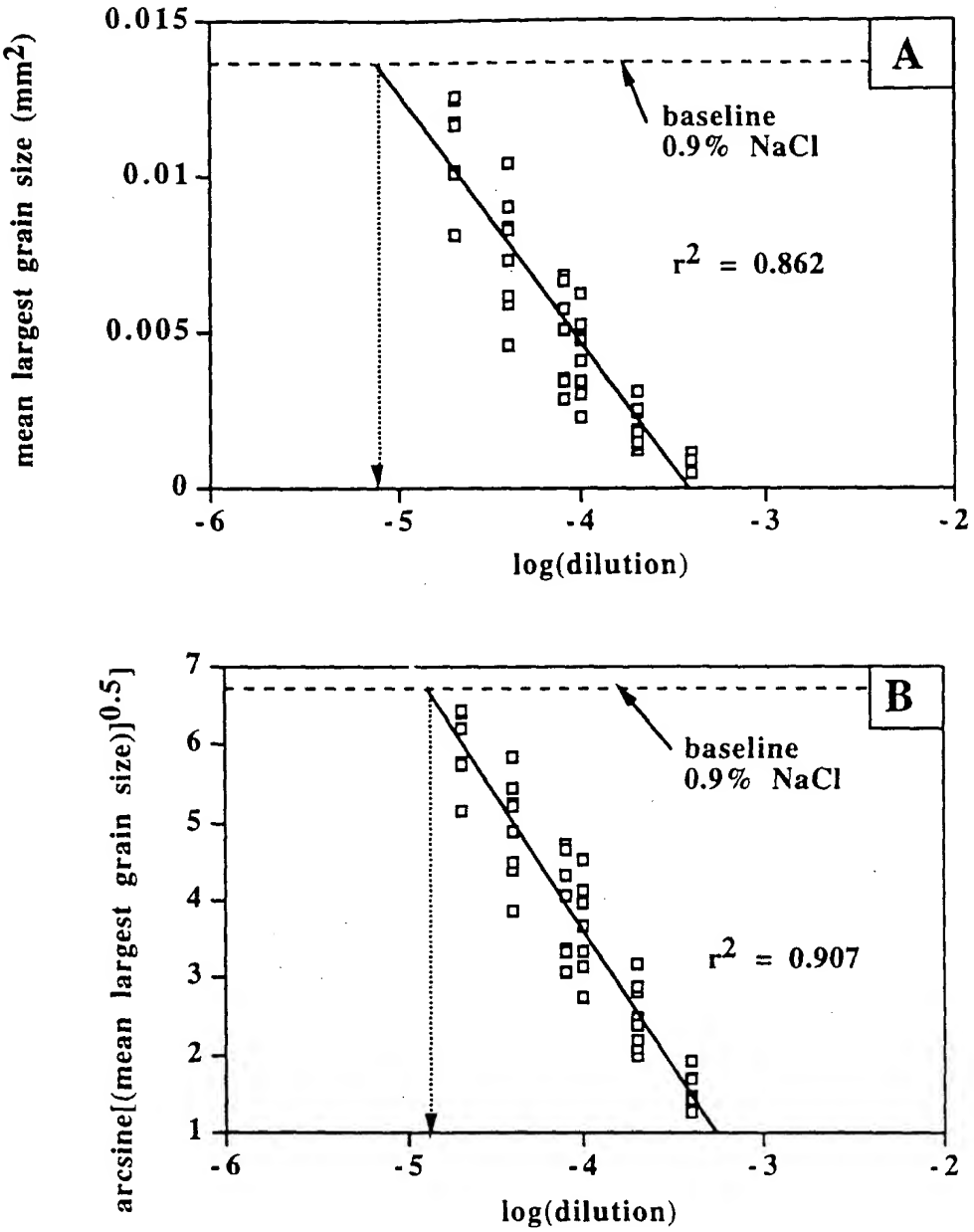

A
0.015
0.01
0.005
0
mean largest grain size (mm²)
baseline
0.9% NaCl
r² = 0.862
-6
-5
-4
-3
-2
log(dilution)
B
7
6
5
4
3
2
1
arcsine[(mean largest grain size)]^0.5]
baseline
0.9% NaCl
r² = 0.907
-6
-5
-4
-3
-2
log(dilution)

FIG. 8.17

EP 2 096 434 A1

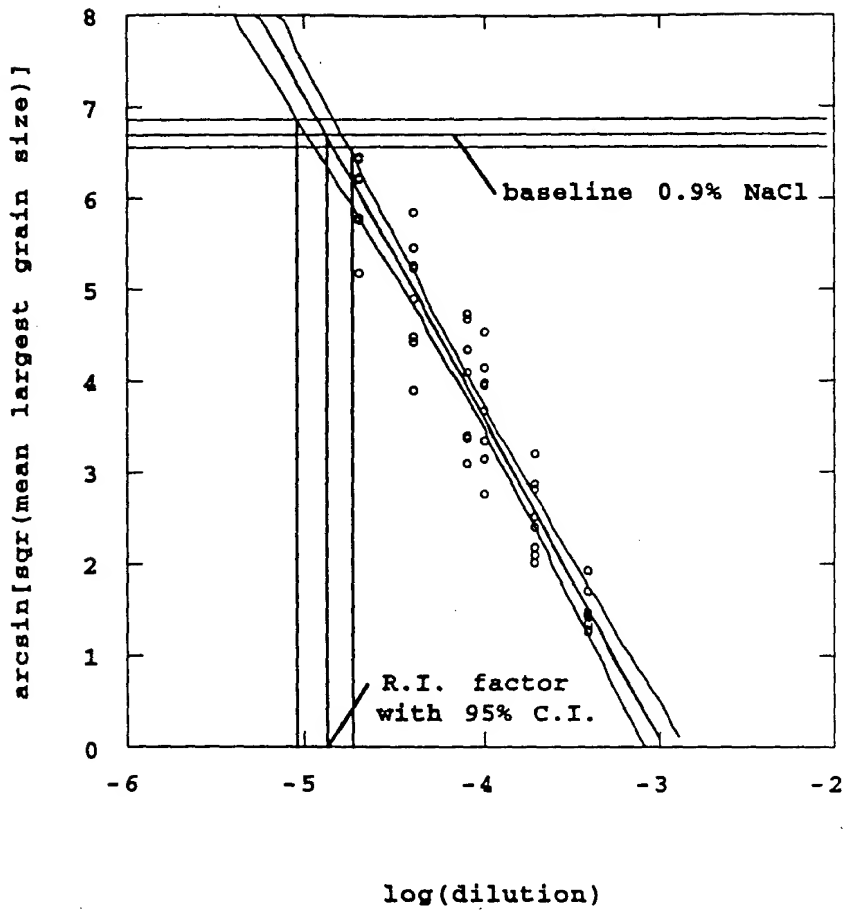



FIG. 8.18

FO2030-8465960

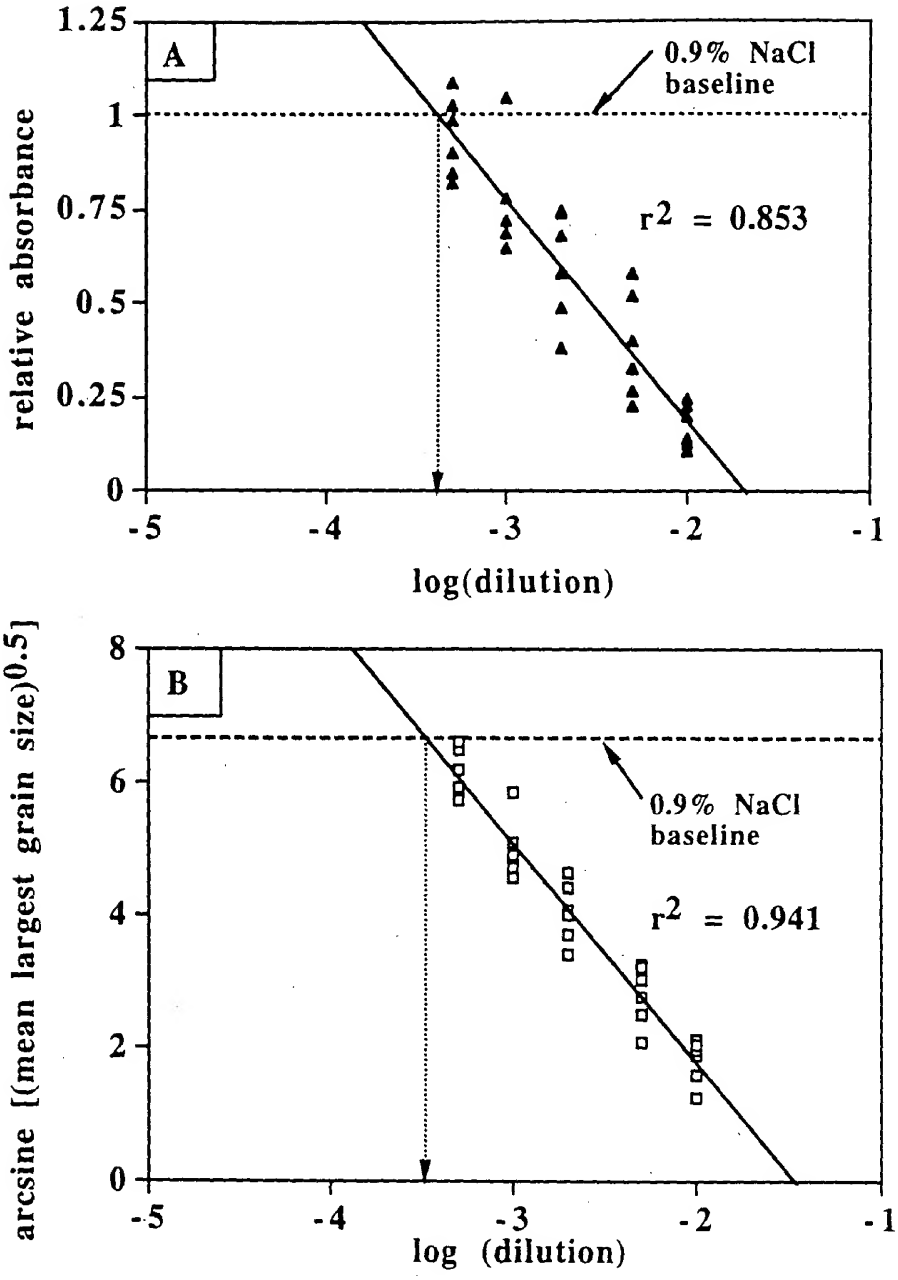



FIG. 8.19

EP 2 969 058 B1

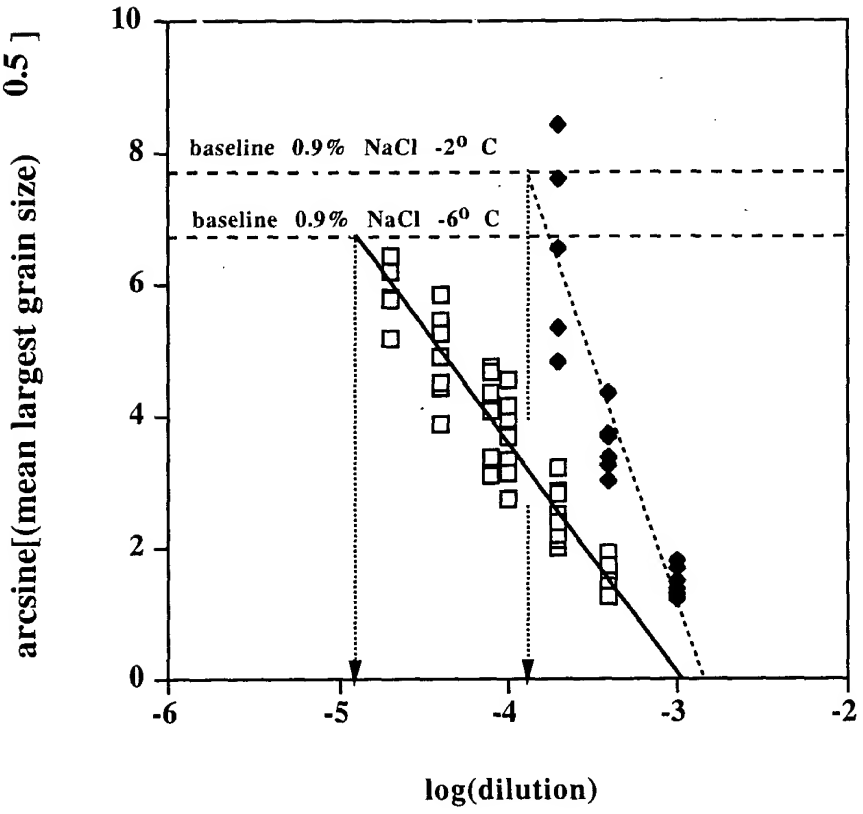



FIG. 8.20

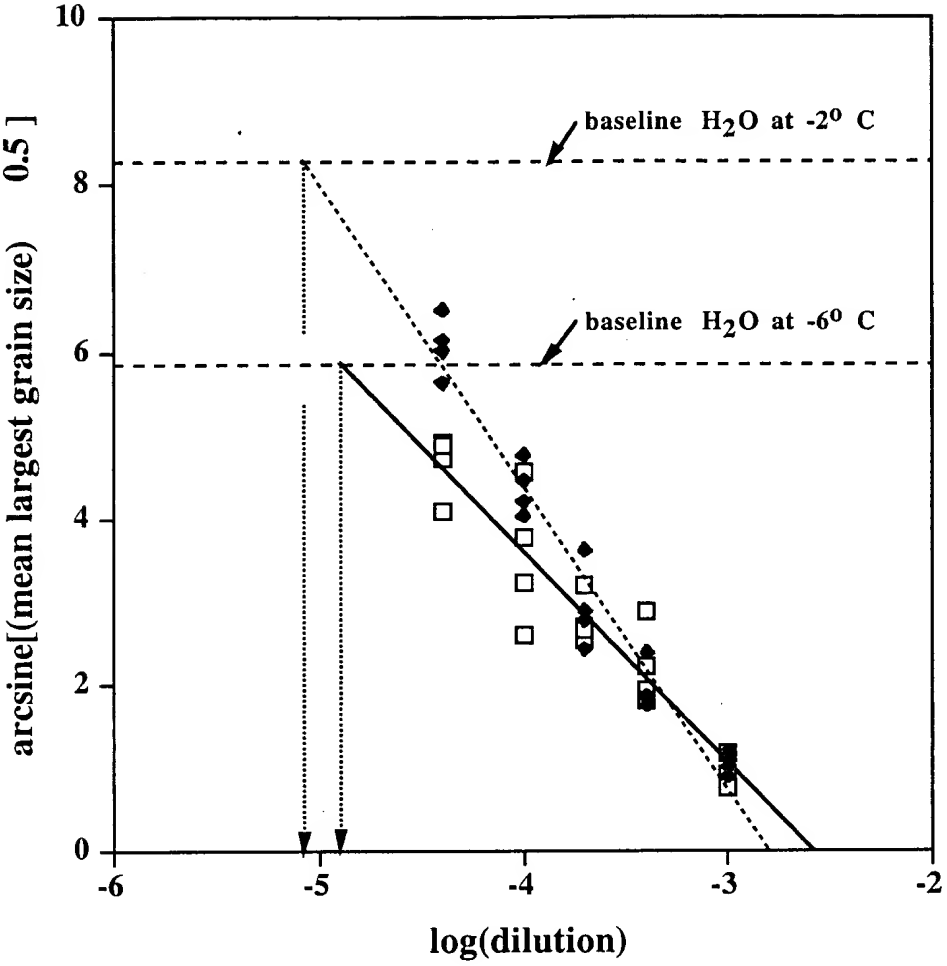

10
8
6
4
2
0
-6
-5
-4
-3
-2
baseline $H_2O$ at -2° C
baseline $H_2O$ at -6° C
arcsine[(mean largest grain size) 0.5 ]
log(dilution)

FIG. 8.21

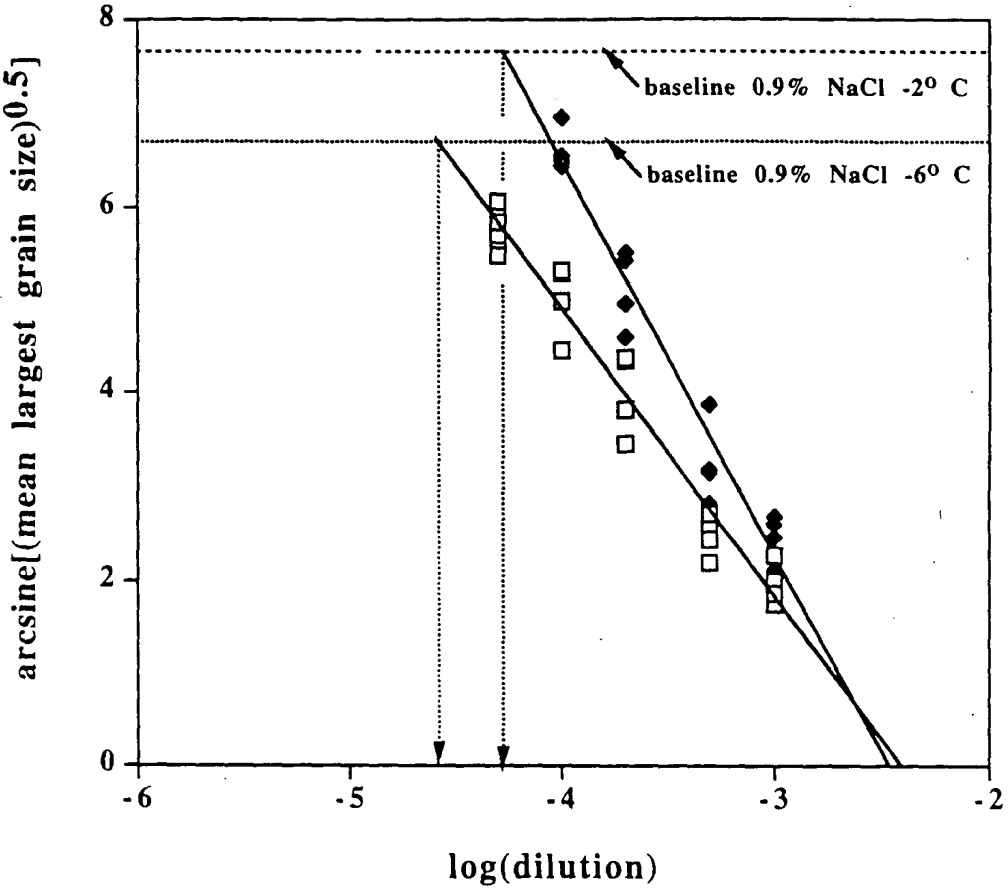

8
6
4
2
0
-6
-5
-4
-3
-2
baseline 0.9% NaCl -2° C
baseline 0.9% NaCl -6° C
arcsine[(mean largest grain size)0.5]
log(dilution)

FIG. 8.22

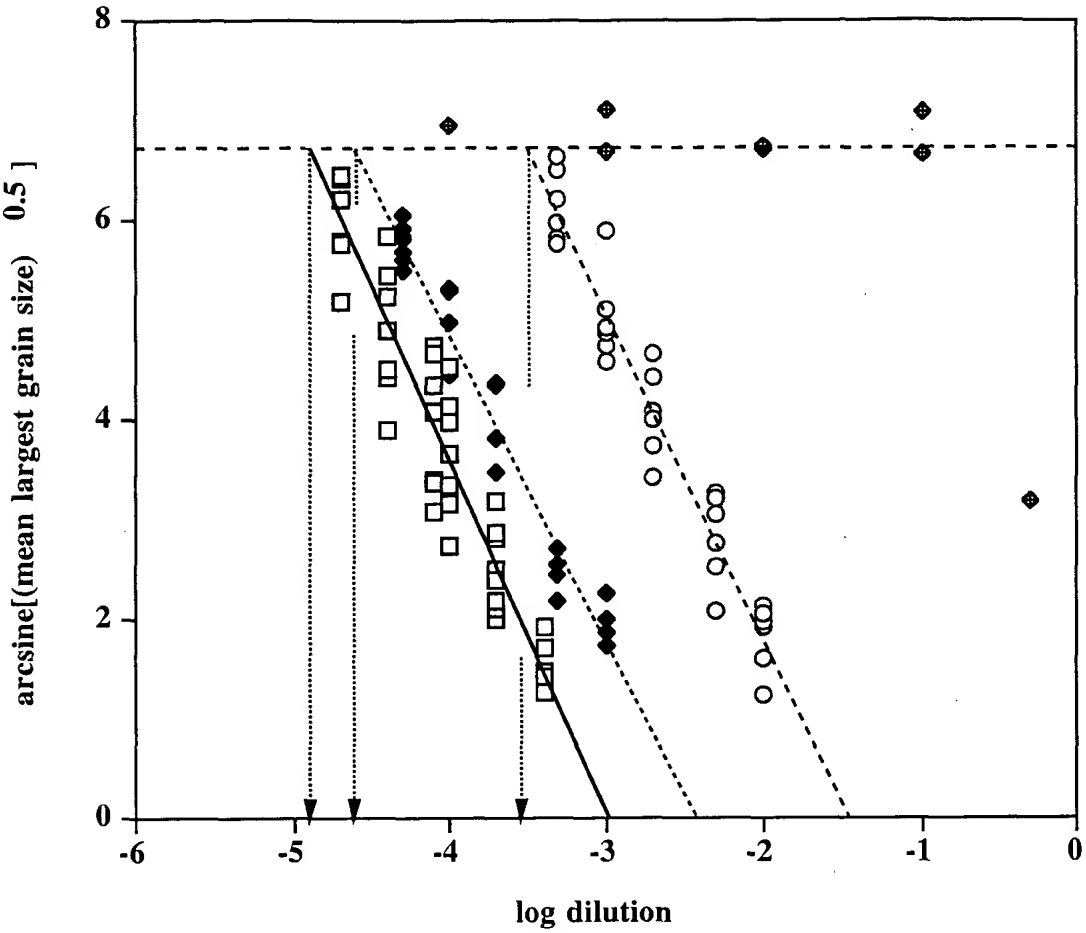

arcsine[(mean largest grain size) 0.5 ]
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
log dilution

FIG. 8.23

T0Z090 · 8tE9Z86O

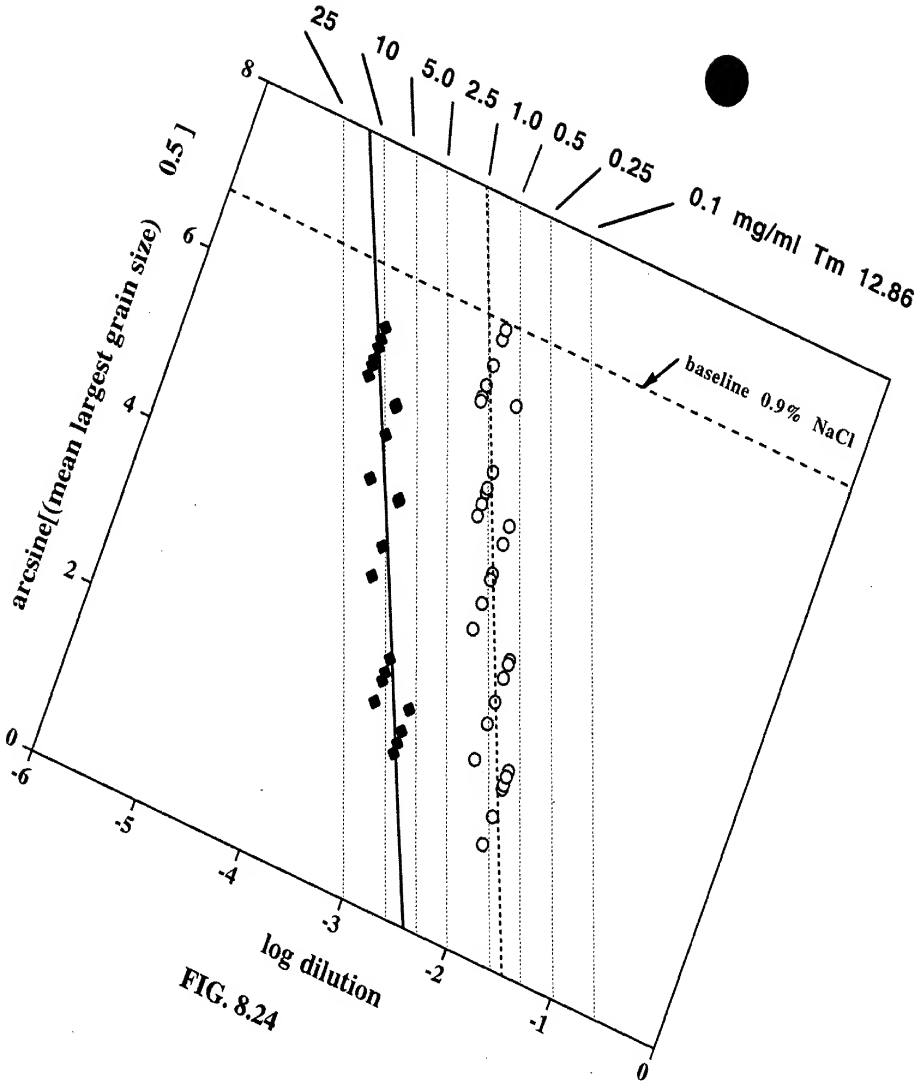



FIG. 8.24

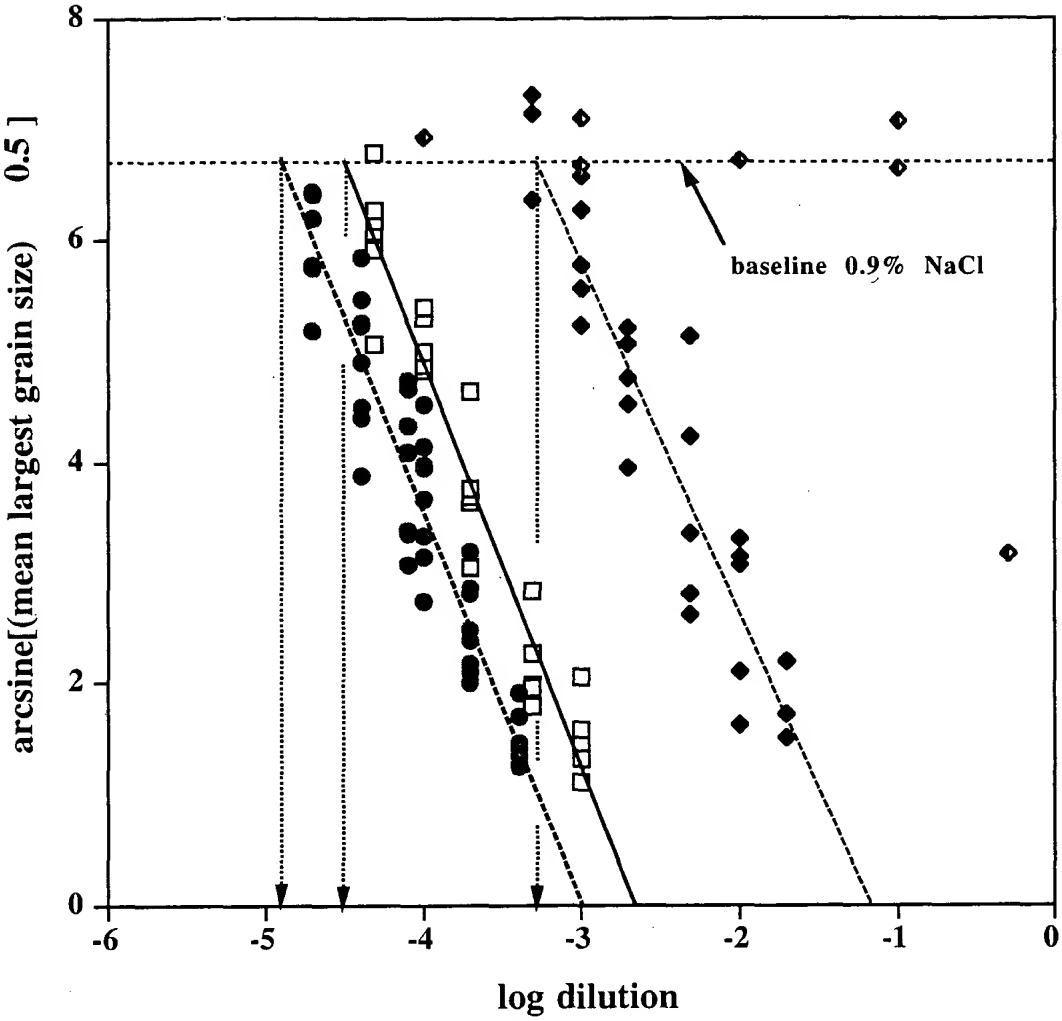

8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
arcsine[(mean largest grain size) 0.5 ]
log dilution
baseline 0.9% NaCl

FIG. 8.25

T0/090 "9t£9/960

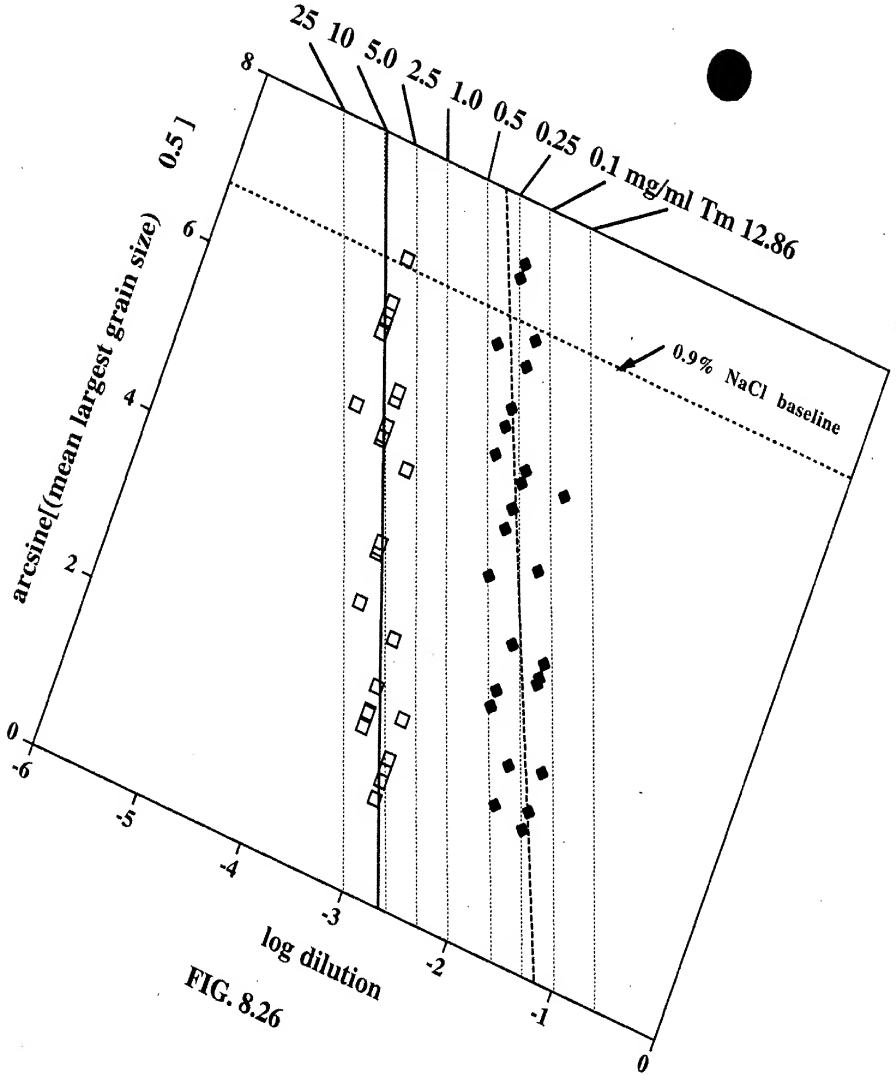



FIG. 8.26

T0270901 - 04E56860

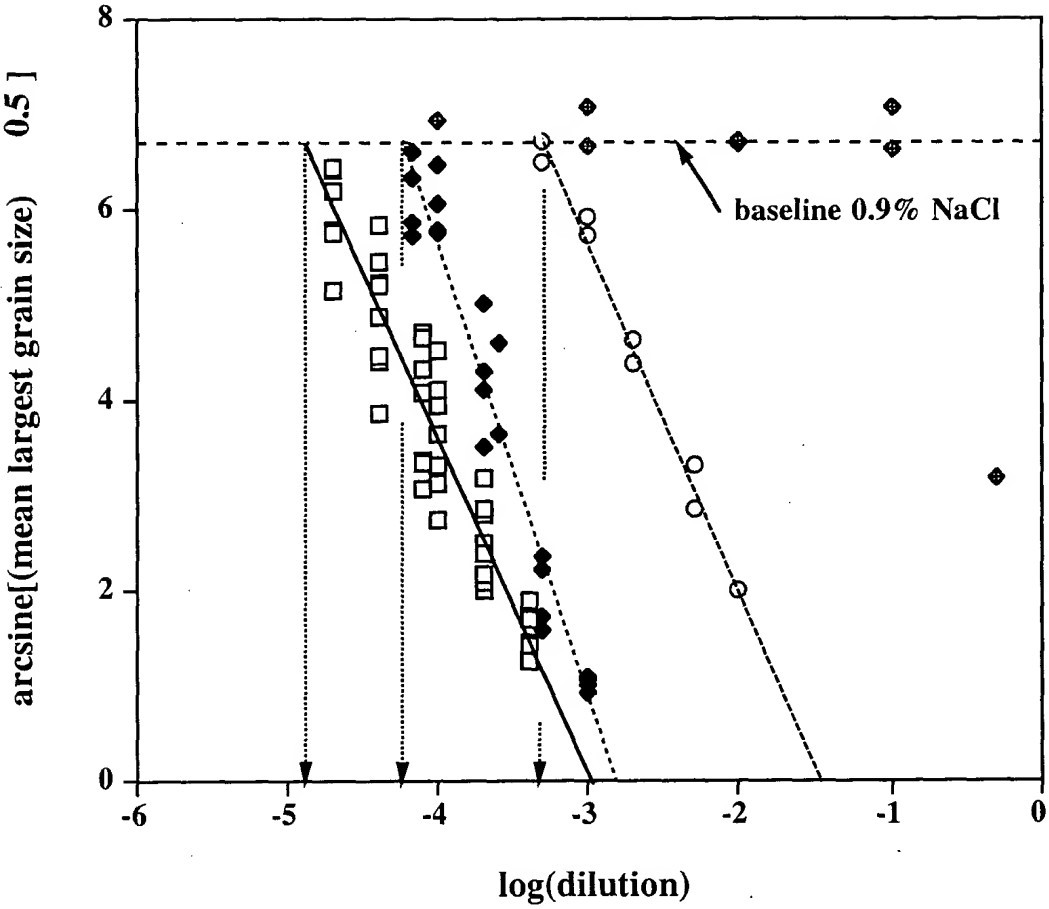



FIG. 8.27

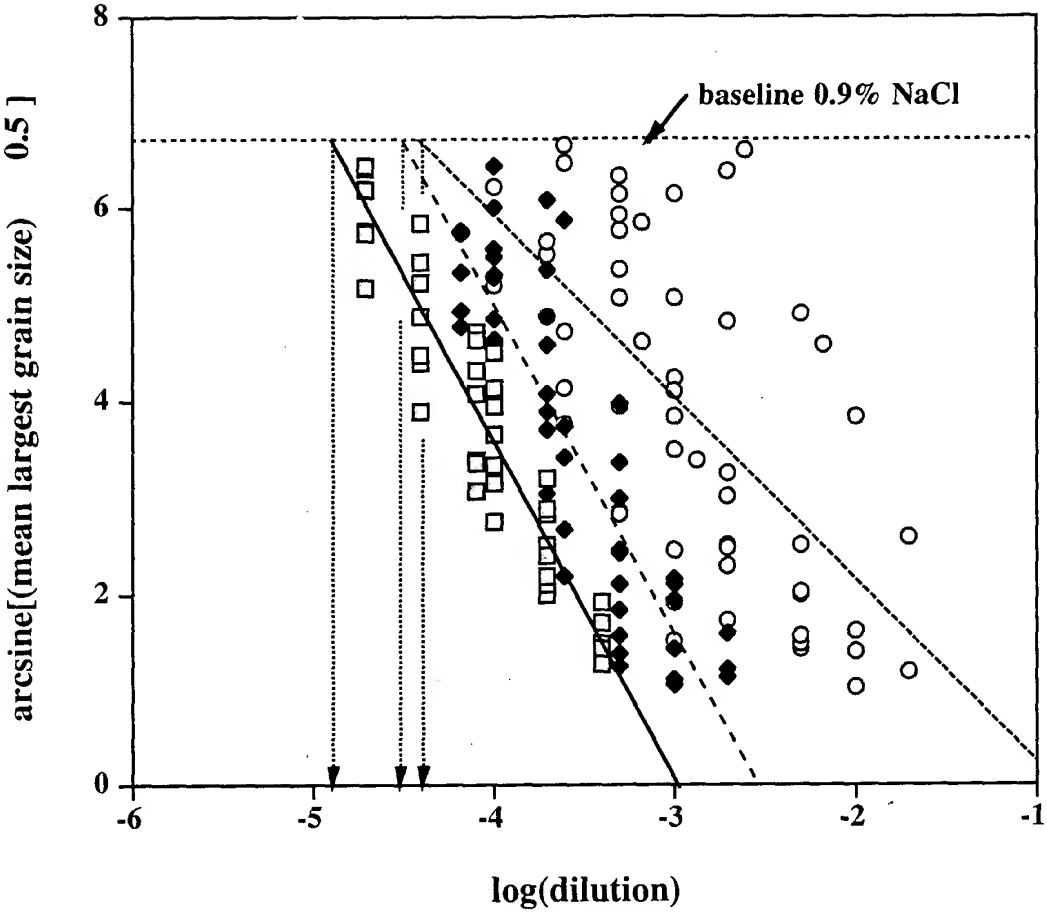

baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5 ]
log(dilution)
8
6
4
2
0
-6
-5
-4
-3
-2
-1

FIG. 8.28

TP-2593-84259360

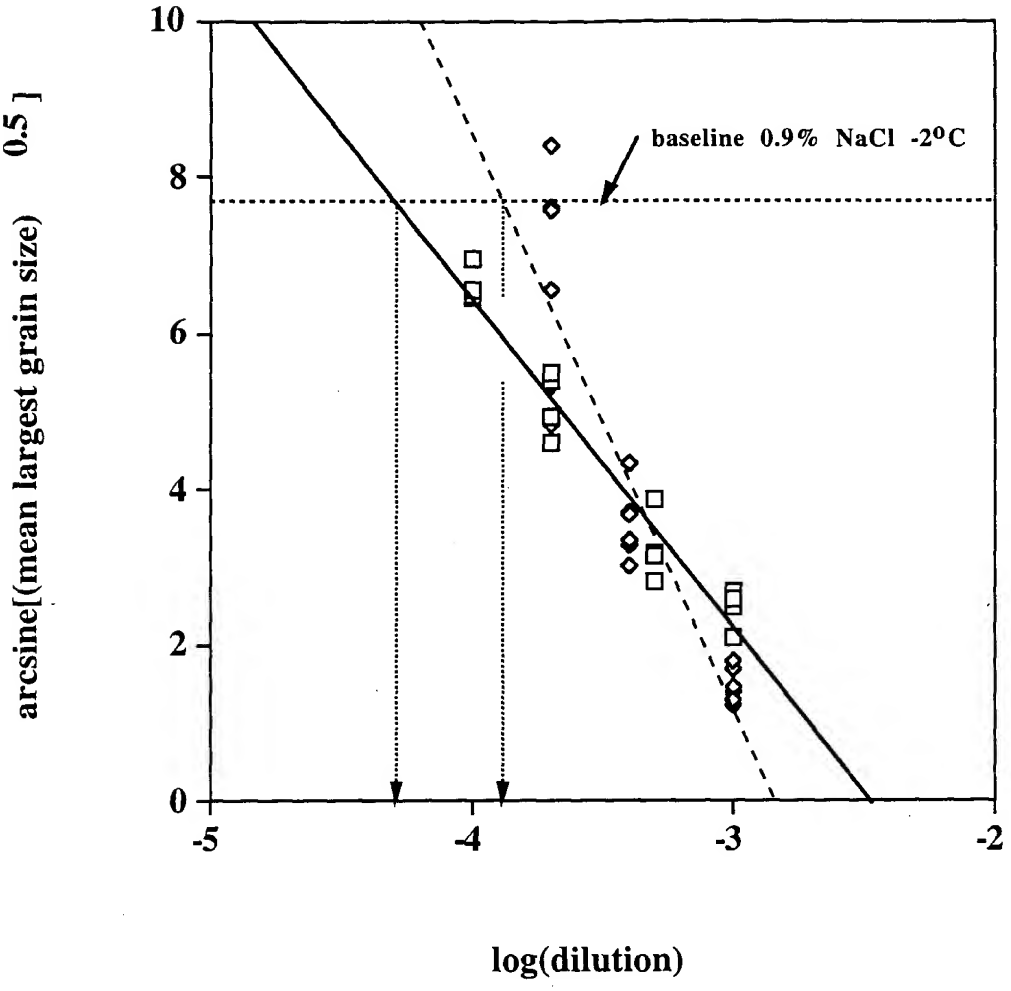



FIG. 8.29

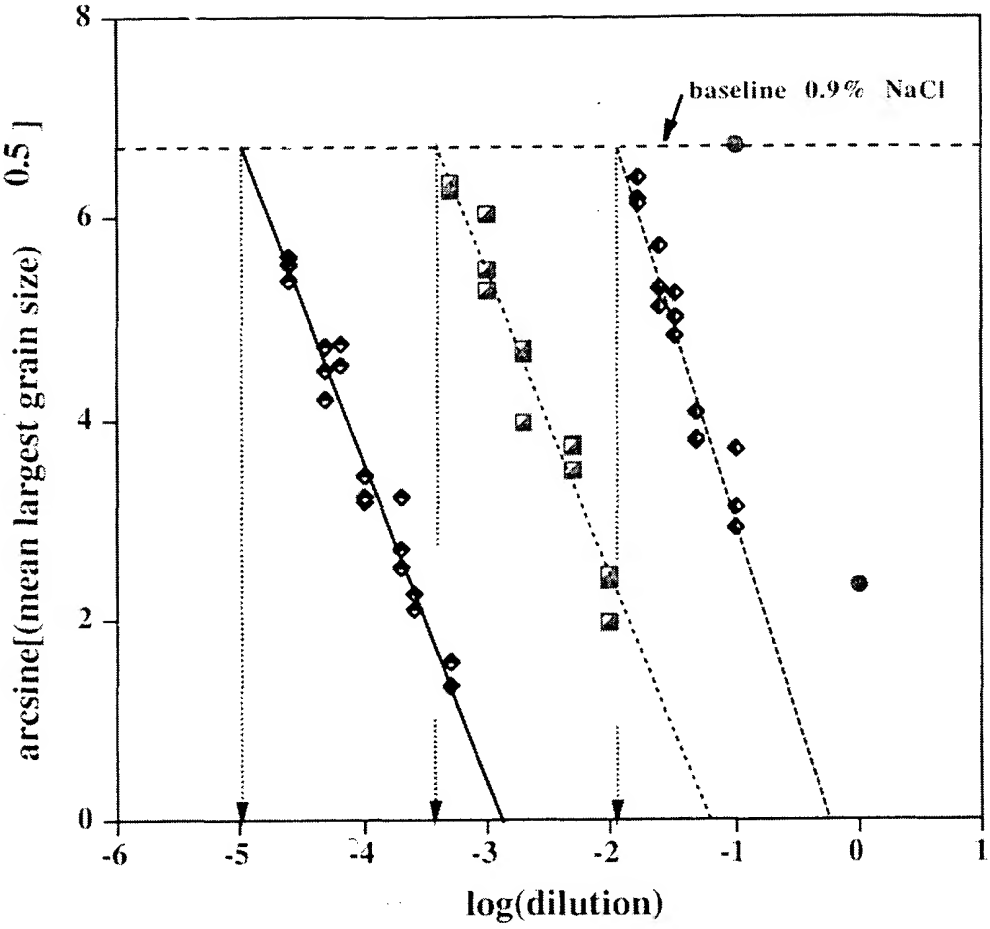



FIG. 8.30

EP 2 950 842 B1

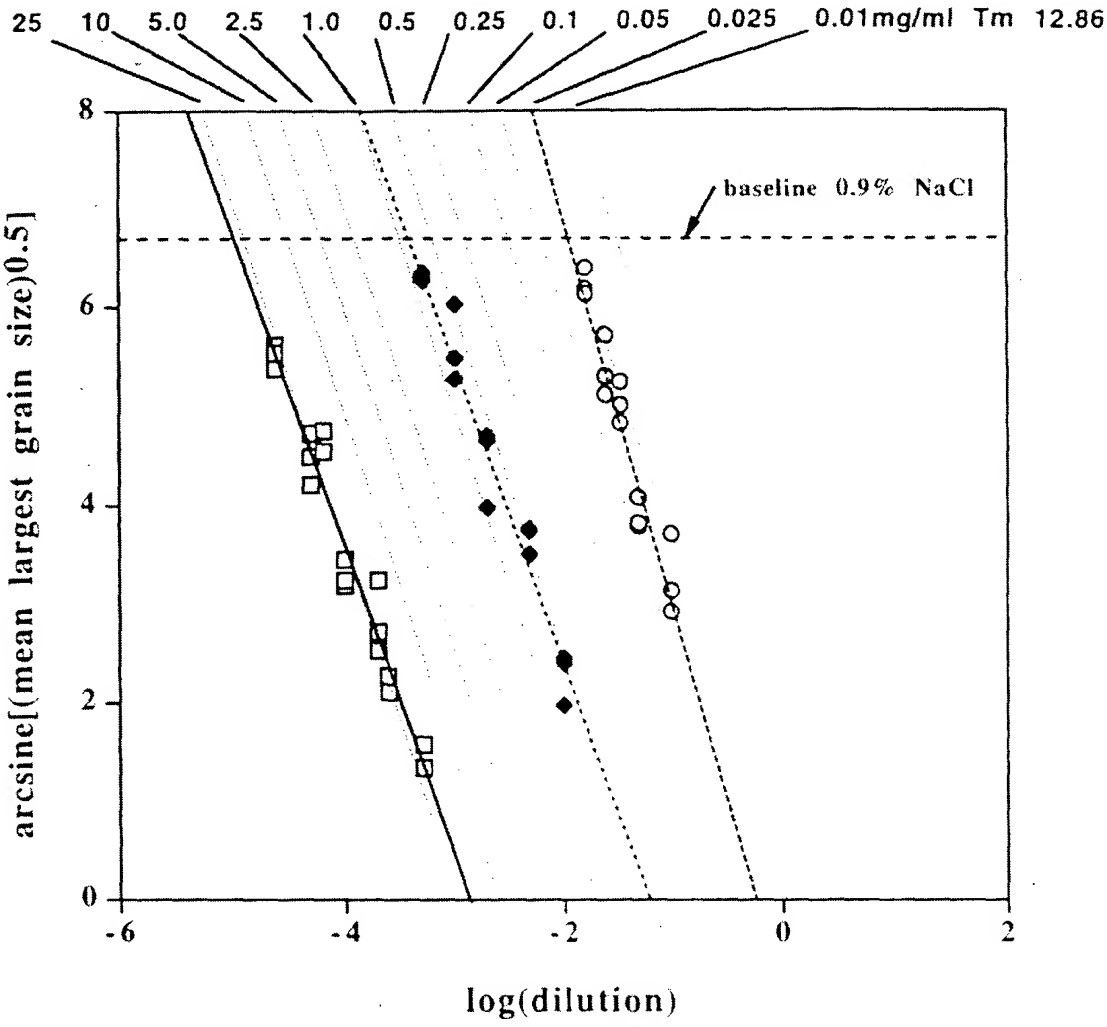



FIG. 8.31

TO2005084629A2

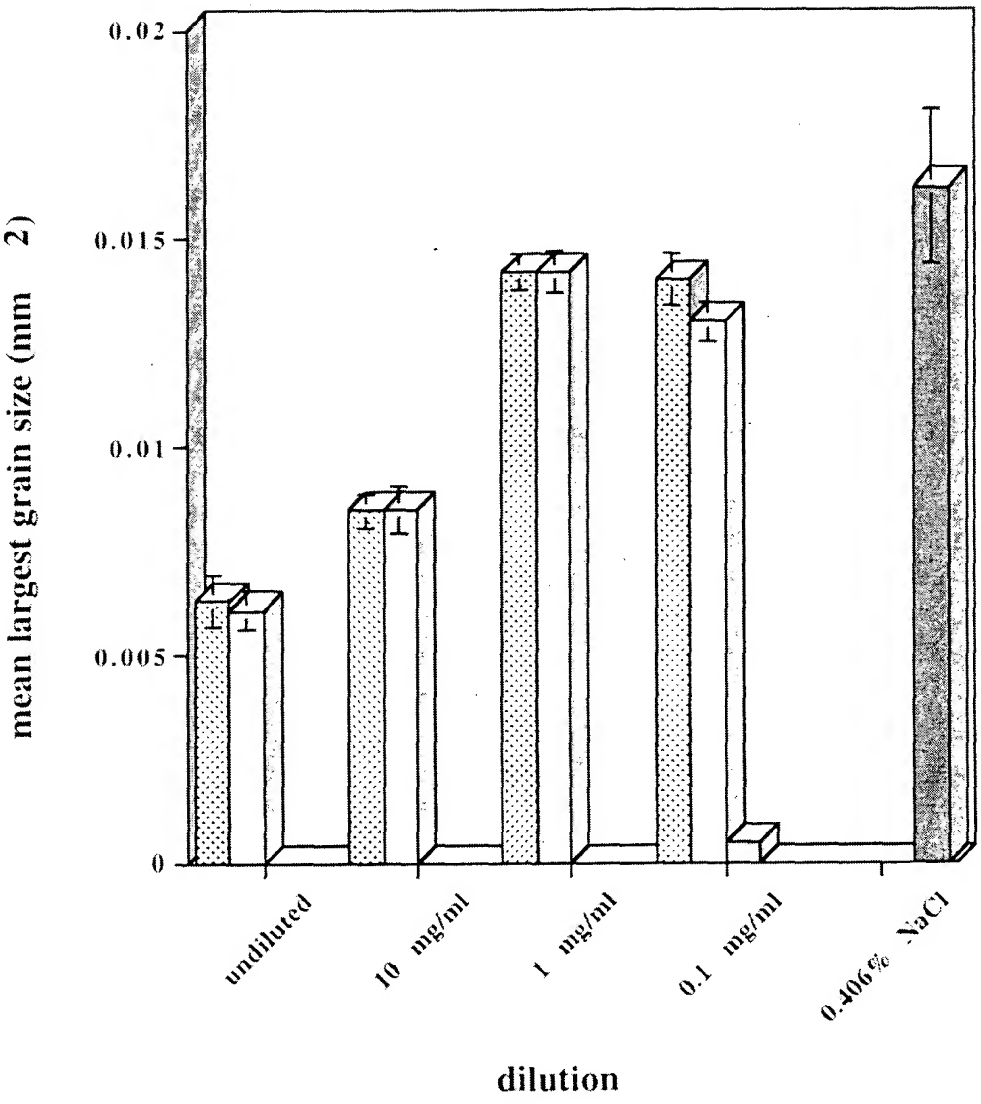

0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
undiluted
10 mg/ml
1 mg/ml
0.1 mg/ml
0.406% NaCl
dilution

FIG. 8.32

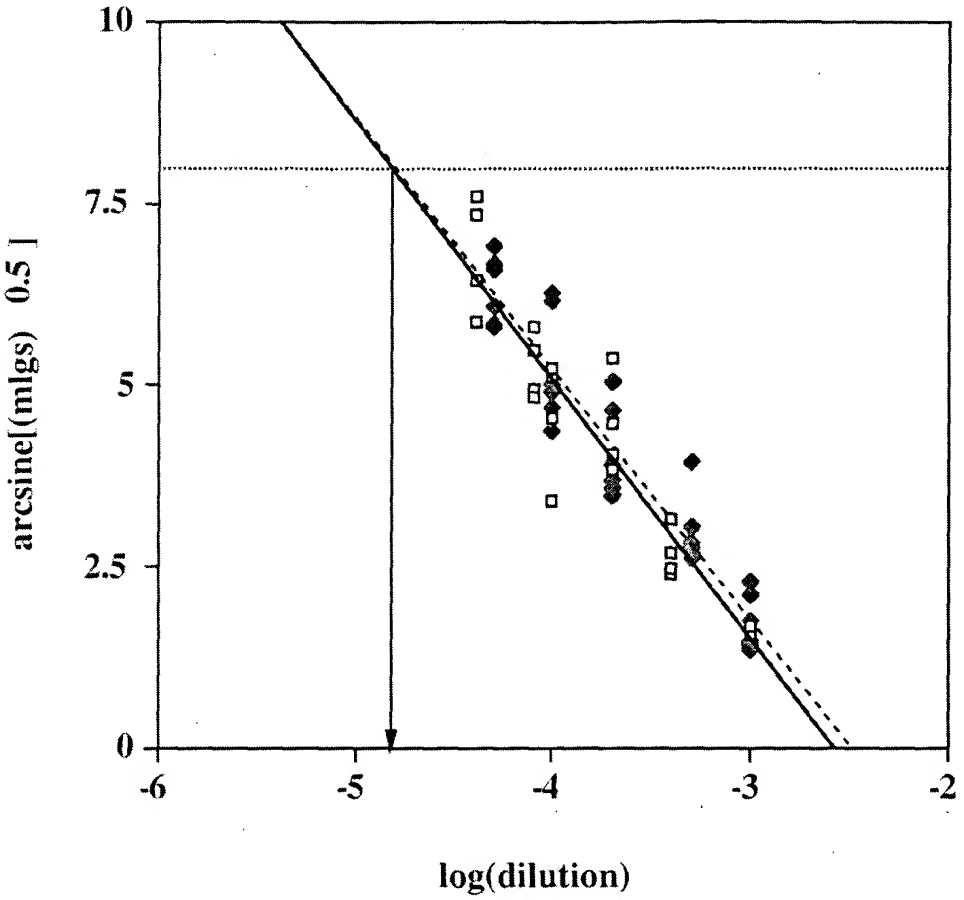



FIG. 8.33

TD 2050 - 84 - 25 - 2850

TO2006-0802-0TE2426050

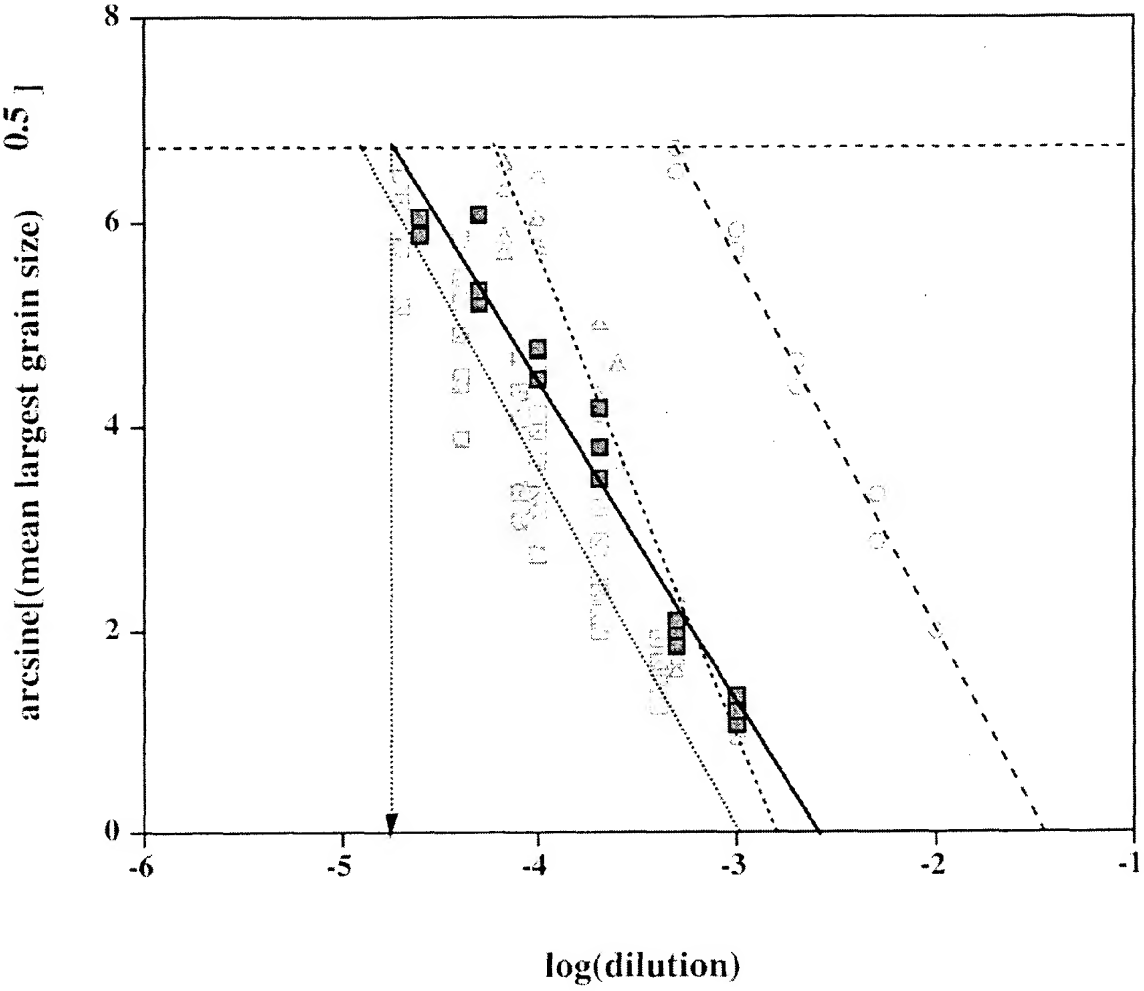



FIG. 8.34

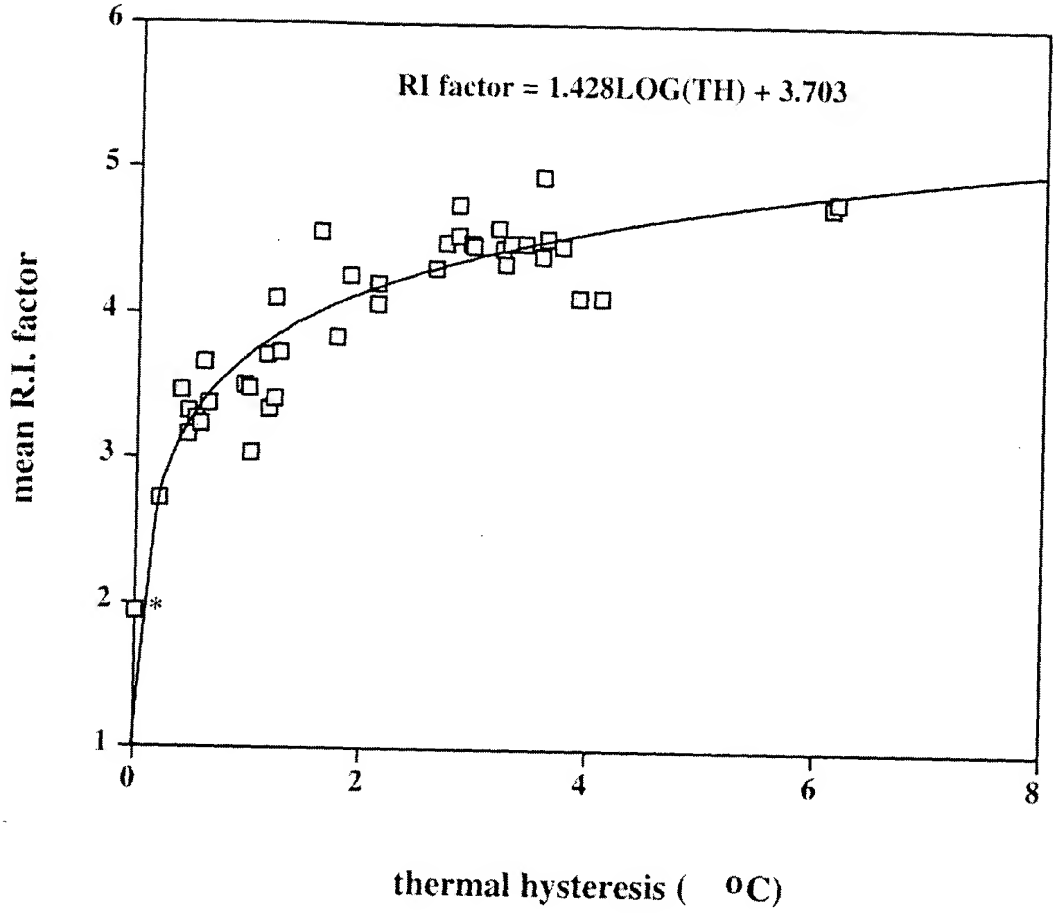

RI factor = 1.428LOG(TH) + 3.703
mean R.I. factor
thermal hysteresis ( °C)
6
5
4
3
2
1
0
2
4
6
8
*

FIG. 8.35

EP 0 250 847 B1

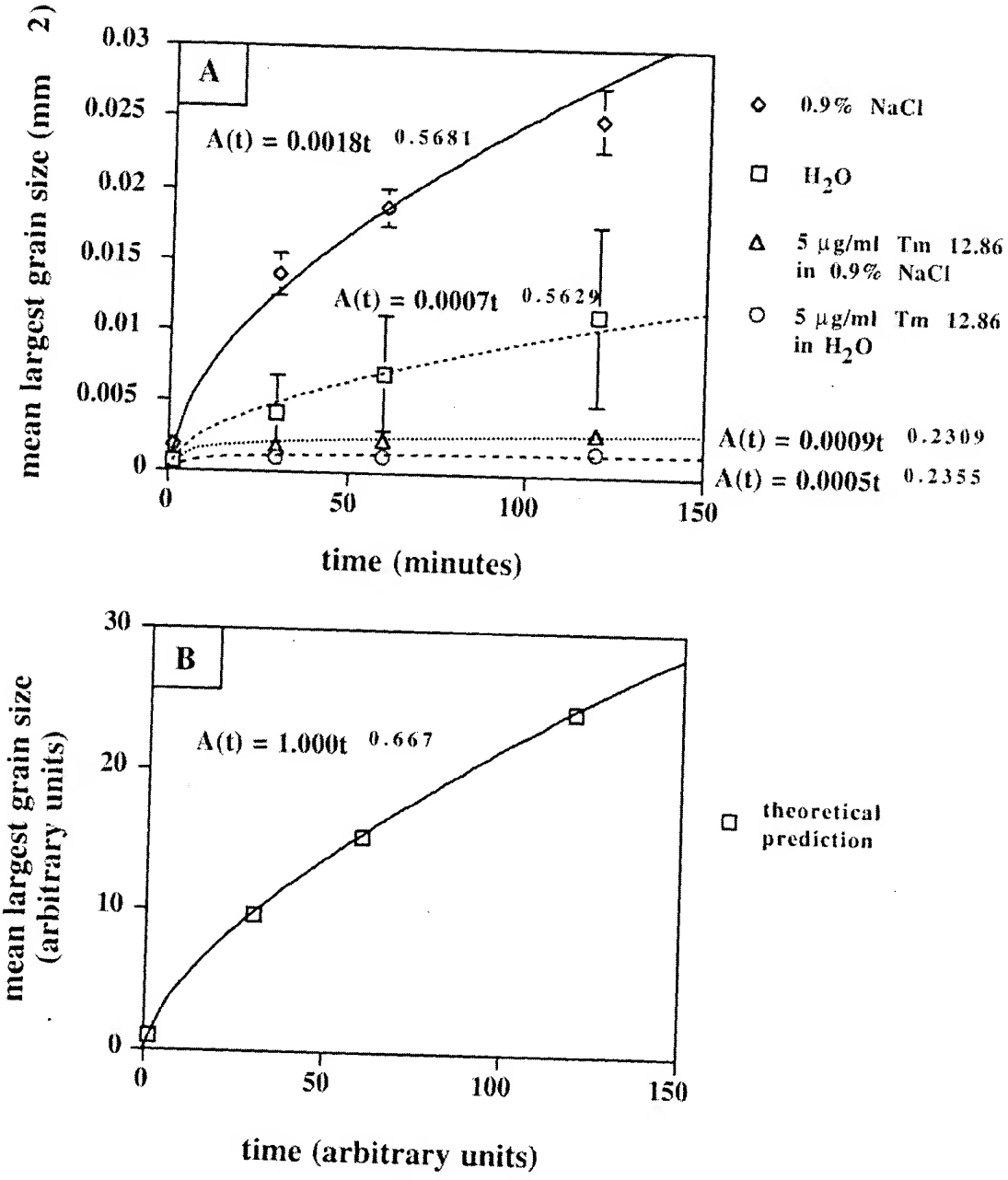



FIG. 8.36

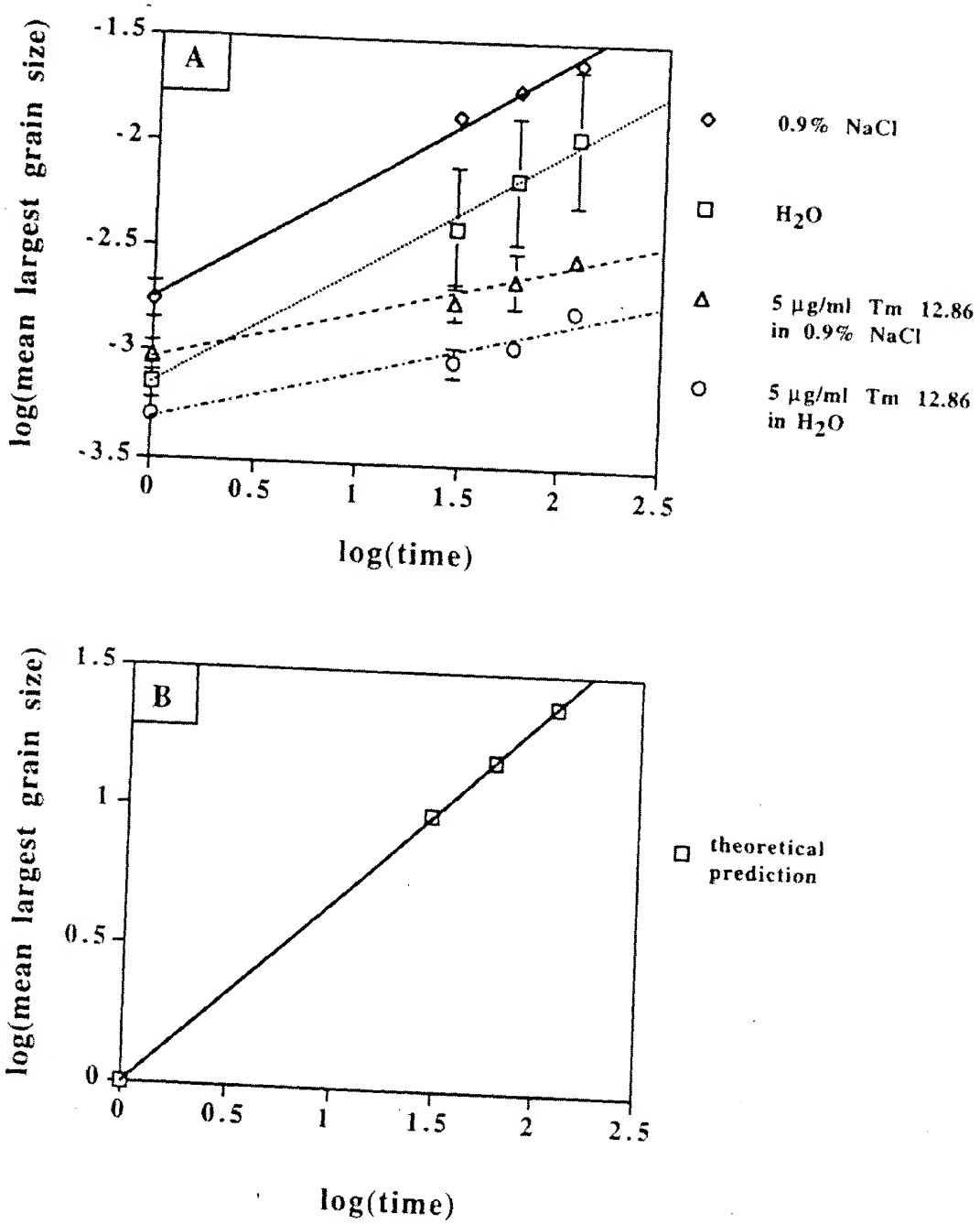
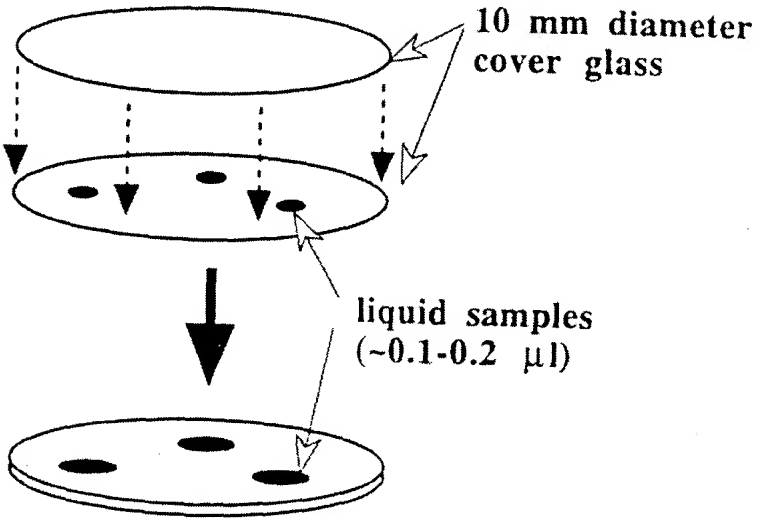

A
log(mean largest grain size)
-1.5
-2
-2.5
-3
-3.5
0
0.5
1
1.5
2
2.5
log(time)
0.9% NaCl
$H_2O$
5 µg/ml Tm 12.86 in 0.9% NaCl
5 µg/ml Tm 12.86 in $H_2O$
B
log(mean largest grain size)
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
log(time)
theoretical prediction

FIG. 8.37

# "Sandwich" method of R.I. assessment

1.




2.

3. FREEZE ON ~-80 C
ALUMINUM PLATE (~10 MIN.)

4. PLACE ON COLD STAGE,
ANNEAL AT -6 C UP TO
12+ HOURS

FIG. 8.38

Patent-6,096,549

US 2003/0194368 A1

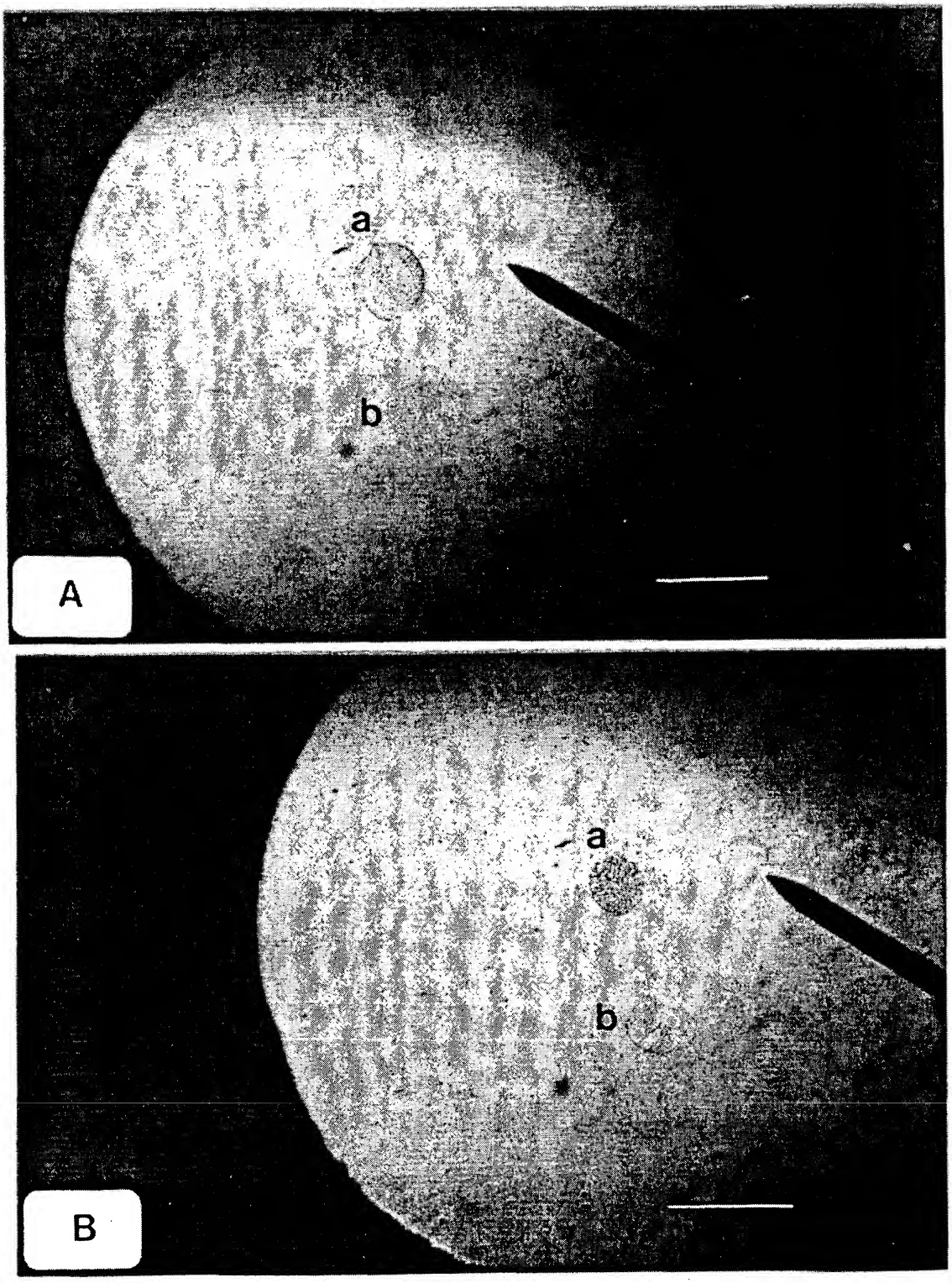



FIG. 8.39

EP 2 705 845 A1 09-07-2014

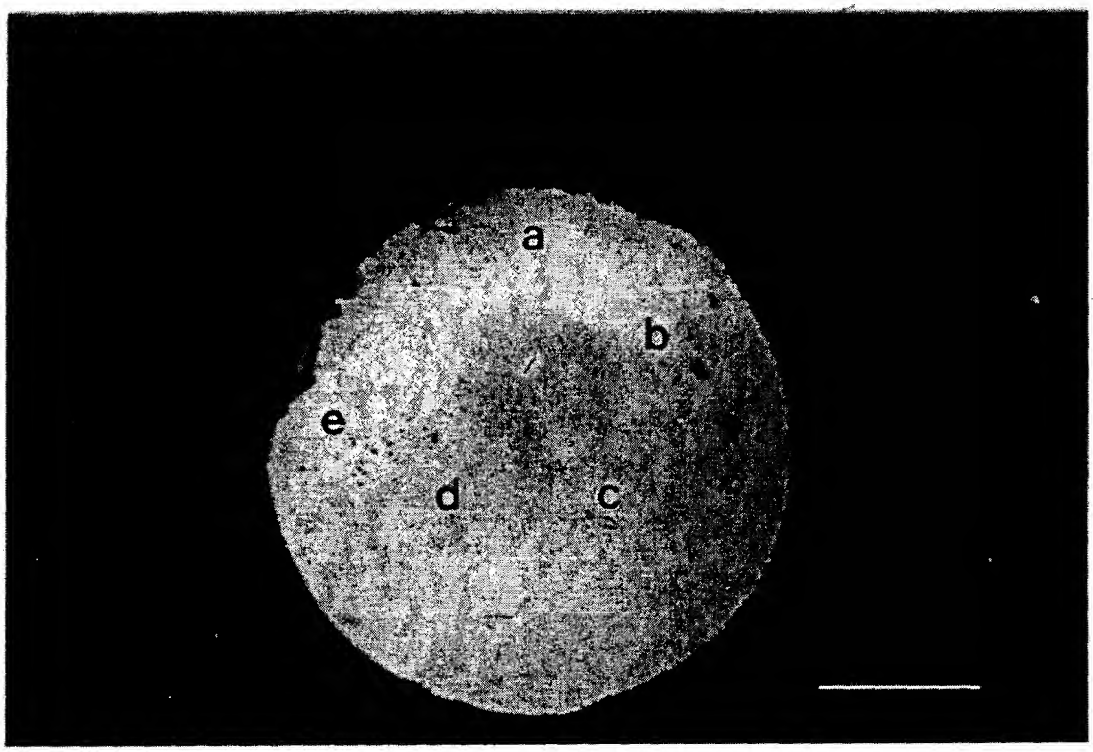



FIG. 8.40

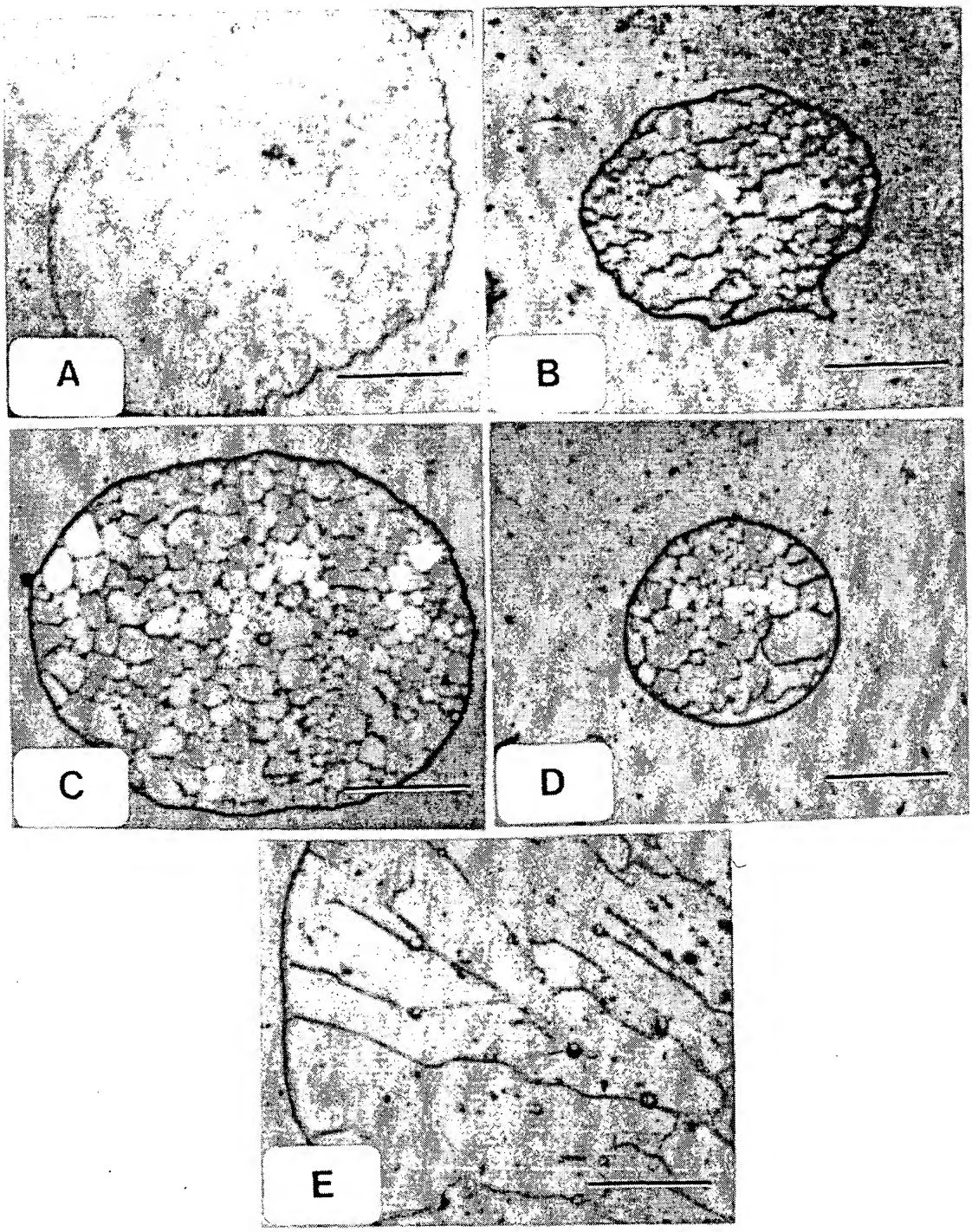



FIG. 8.41

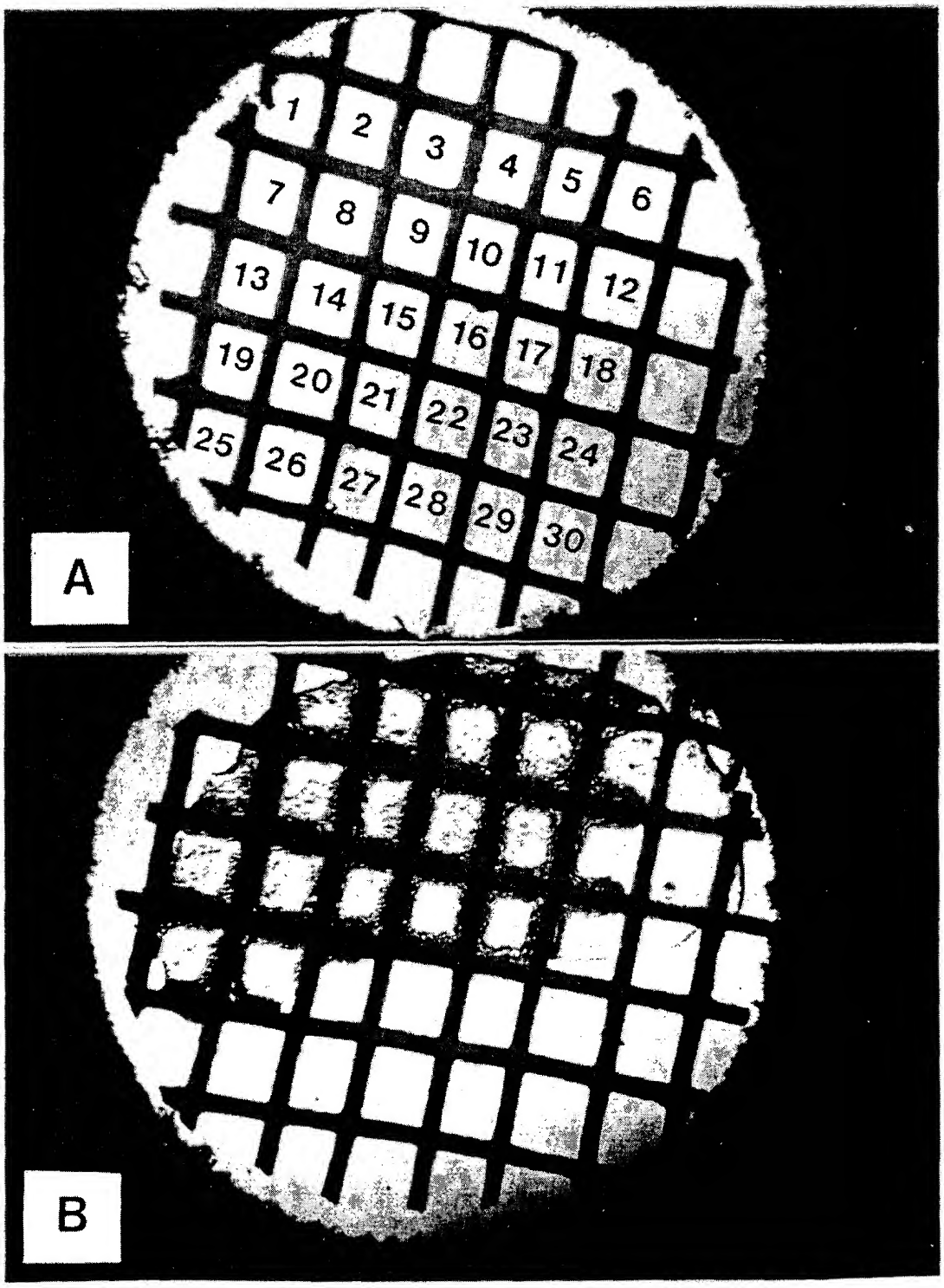

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
A
B

FIG. 8.42

# DNA sequence of Tm 13.17 cDNA clone

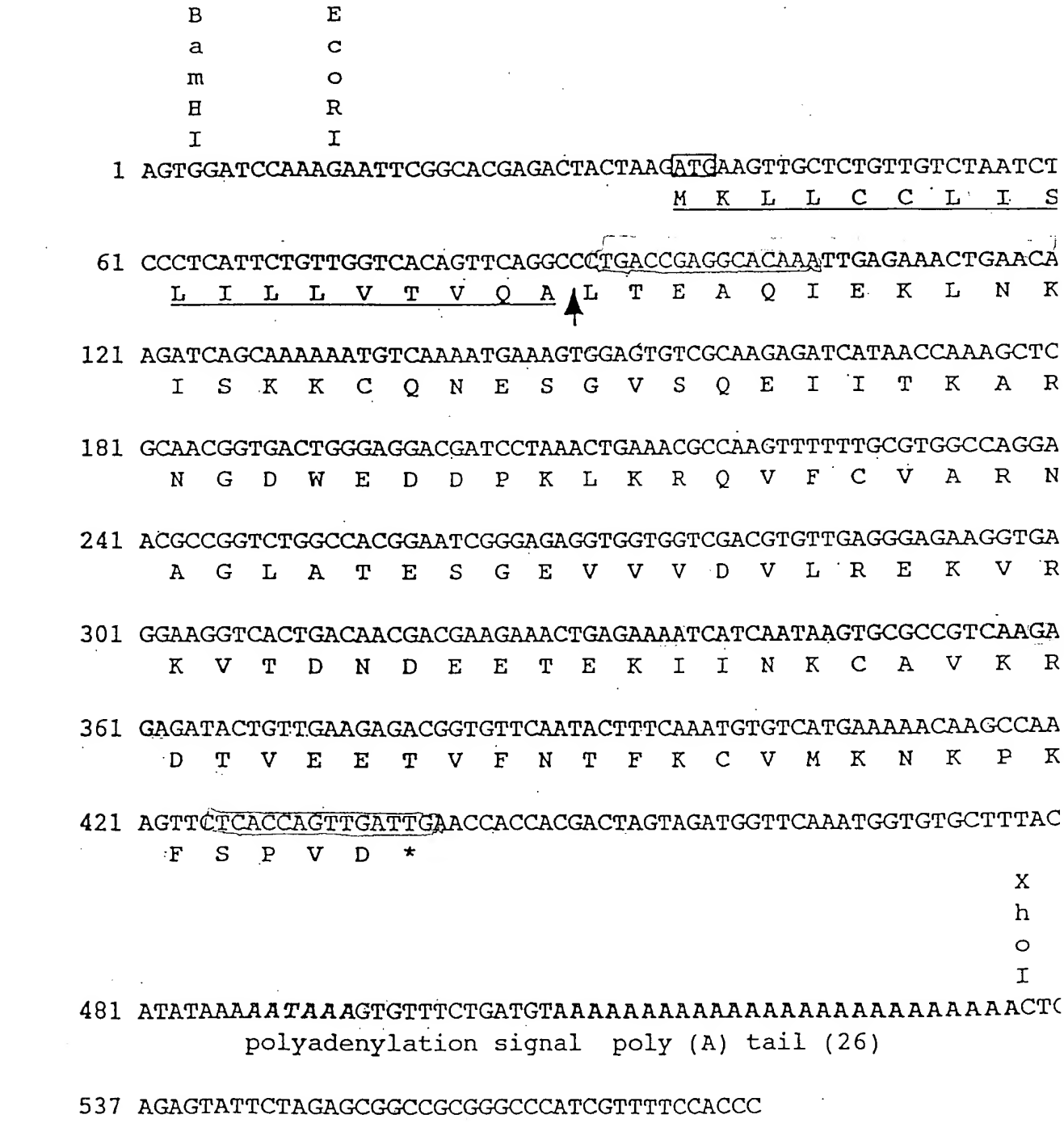
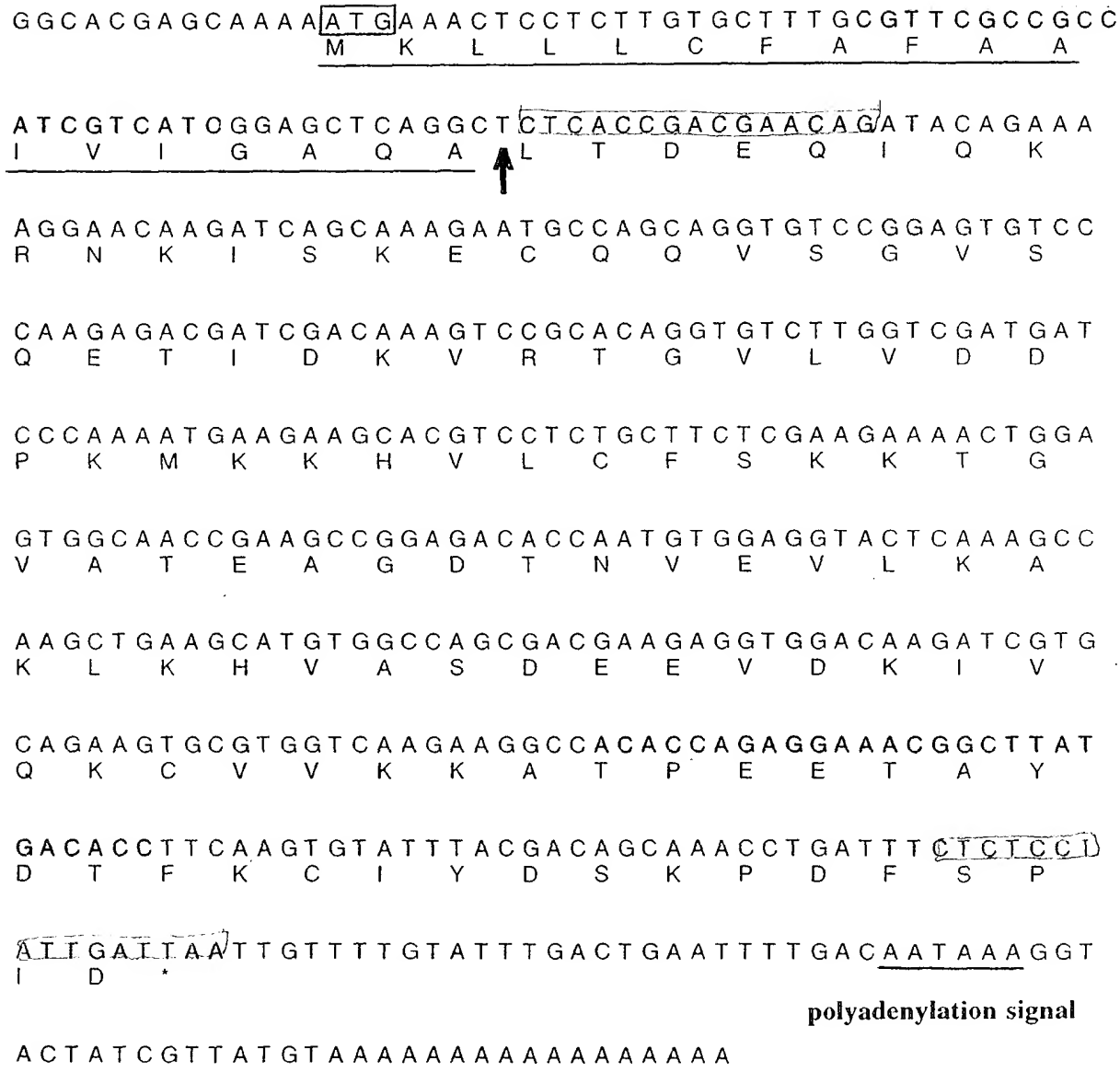



**FIG. 8.43**

PCT/US93/04503




poly (A) tail

**FIG. 8.44**